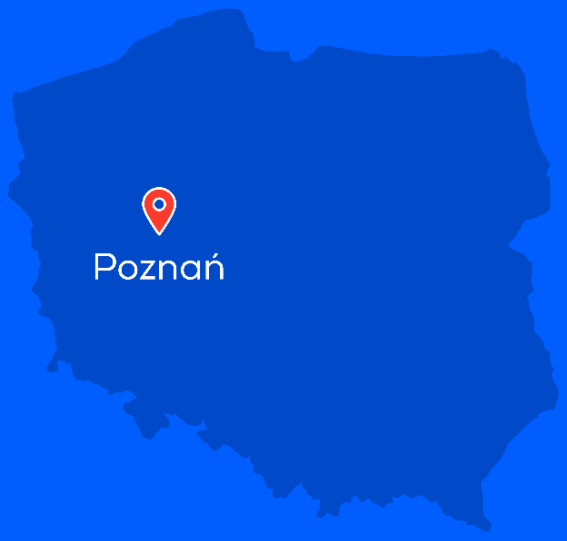
Second Polish RNA Biology Meeting



24-27 June, 2025

Website

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Day 1, Tuesday, June 24

15:00 – 17:00 Registration

17:00 – 17:15 Opening

17:15 – 18:00 Keynote Lecture

Chair: **Krzysztof Sobczak**, Adam Mickiewicz University in Poznań, Poznań, Poland

L1 **Ramesh Pillai**, Department of Molecular Biology, University of Geneva, Switzerland

RNA modifications in control of mammalian gene expression

18:00 – 18:45 Keynote Lecture

Chair: **Gracjan Michlewski**, International Institute of Molecular and Cell Biology in Warsaw, Poland

L2 **Sebastian Glatt**, Małopolska Center of Biotechnology, Kraków, Poland

tRNAslational control of eukaryotic gene expression

From 19:00 Get together party

L1

RNA modifications in control of mammalian gene expression

Ramesh S. Pillai

Department of Molecular Biology, University of Geneva, 30 Quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland.

N^6-methyladenosine (m^{6}A) is an essential internal RNA modification that is critical for gene expression control in most organisms. They are catalyzed by RNA methyltransferases 'writers' on specific targets, while protein 'readers' with a YTH domain recognize the m^{6}A marks to mediate molecular functions like RNA splicing, mRNA decay and translation control. The functional relevance of these marks is demonstrated by the ability of RNA demethylase 'erasers' to remove this mark, pointing to potential reversibility and regulation. I will report the recent studies from the lab on the writer human METTL16 to reveal its physiological roles and the mechanistic basis for target regulation. We use a combination of mouse genetics and biochemistry to decipher how the targets are engaged and how this impacts developmental programs. Our results highlight the pivotal role of an m^{6}A RNA methyltransferase in facilitating early developmental decisions via regulation of SAM availability.

L2

tRNAslational control of eukaryotic gene expression

Sebastian Glatt

Małopolska Center of Biotechnology, Kraków, Poland

My Research Group studies different translation control mechanisms, which regulate the production of specific sets of proteins by chemical modifications of tRNA molecules. Every protein in the cell is produced by the ribosome, which uses transfer RNA (tRNA) molecules to translate the sequence information coded in mRNAs into correctly assembled poly-peptide chains. The lab is focusing on understanding the molecular mechanisms that lead to the specific base modifications in anticodons of tRNAs. These modifications have a strong influence on the efficiency and accuracy of the codon-anticodon pairing and therefore regulate the translational rates and folding dynamics of protein synthesis. Recent findings have shown that alterations of these modification pathways play important roles in the onset of certain neurodegenerative diseases and cancer. We mainly use X-ray crystallography (MX) and cryogenic electron microscopy (cryo-EM) to obtain snapshots of the involved macromolecular machines and analyse their reaction intermediates at atomic resolution. Subsequently, we employ different complementary in vitro and in vivo approaches to validate and challenge our structural observations. We also have started working on other (t)RNA modification pathways and elucidate the structure of folded RNA molecules directly by cryo-EM. Furthermore, we aim to understand how these post-transcriptional modifications affect ribosomal decoding and translation elongation by directly imaging translating ribosomes at atomic resolution. Last but not least, we develop novel structural, biochemical and biophysical approaches to study structured RNA domains. In summary, our work contributes to the fundamental understanding of eukaryotic gene expression and its complex regulatory mechanisms.

Day 2, Wednesday, June 25

08:00 - 09:00 Registration

09:00 - 9:45 Keynote Lecture

Chair: **Kinga Kamieniarz-Gdula**, Adam Mickiewicz University in Poznań, Poznań, Poland

L3 **Andrzej Dziembowski**, International Institute of Molecular and Cell Biology in Warsaw, Poland

Complex metabolic pathways of endogenous and therapeutic mRNAs *in vivo*

9:45 - 10:15 Coffee break

Morning Session

10:15 - 12:00 Session on **RNA and Disease**

Chair: **Magdalena Dziembowska**

T1 **Daria Niewiadomska**, Adam Mickiewicz University in Poznań, Poznań, Poland

The role of structural elements within mutant mRNA of FMR1 gene containing expanded CGG repeats on the regulation of non-canonical translation of toxic polyglycine protein

T2 **Maria Borja-Gonzalez**, University of Galway, Ireland

miR-199 regulates neuromuscular homeostasis during ageing

T3 **Boris Rogelj**, Jožef Stefan Institute, Ljubljana, Slovenia

C9orf72 mutation alters membrane protein expression in ALS and FTD

T4 **Tomasz Kuliński**, International Institute of Molecular and Cell Biology in Warsaw, Poland

Nuclear RNA decay gone wrong: how DIS3 mutations drive and then escape multiple myeloma

T5 Gracjan Michlewski, International Institute of Molecular and Cell Biology in Warsaw, Poland

5' terminal nucleotide determines the immunogenicity of IVT RNAs

T6 Sandra Fienko, University College London, London, UK

Molecular characterisation of RNA-rich ensembles in Huntington's disease

T7 Agnieszka Fiszer, Institute of Bioorganic Chemistry PAS, Poznań, Poland

HTT loss-of-function contributes to RNA deregulation in developing Huntington's disease neurons

T8 Paweł Sikorski, University of Warsaw, Poland

Effective recognition of double-stranded RNA does not require activation of cellular inflammation

12:00 - 13:00 Lunch

13:00 – 13:45 Keynote Lecture

Chair: **Mikołaj Olejniczak**, Adam Mickiewicz University in Poznań, Poznań, Poland

L4 Kai Papenfort, Friedrich Schiller University of Jena, Germany

From strings of nucleotides to collective behavior: Lessons from Vibrio cholerae and its phages

13:45 – 14:15 Coffee break

Afternoon Session I

14:15 -16:00 Session on **Regulatory RNA**

Chair: **Szymon Świeżewski**, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

T9 Oleg Dmytrenko, Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany

RNA-triggered Cas12a3 cleaves tRNA tails to execute bacterial immunity

T10 **Paulina Jackowiak**, Institute of Bioorganic Chemistry PAS, Poznań, Poland

Knockdown of *Smed ELAC2* in *Schmidtea mediterranea* decreases 5' tiRNA-Gly-GCC levels and reveals its regulatory role in regeneration

T11 **Monika Jóźwiak**, Adam Mickiewicz University in Poznań, Poznań, Poland

The DEAD-box helicases DRH1, RH46 and RH40 remodel the secondary structure of miRNA precursors to regulate miRNA biogenesis in plants

T12 **Halina Pietrykowska**, Adam Mickiewicz University in Poznań, Poznań, Poland

Tiny but mighty: Male-specific MpmiR11889 regulates proper sperm cell development and sexual reproduction efficiency in *Marchantia polymorpha*

T13 **Zbigniew Warkocki**, Institute of Bioorganic Chemistry PAS, Poznań, Poland

Cytoplasmic synthesis of LINE-1 complementary ssDNA via self-primed reverse transcription

T14 **Marta Sztachera**, Institute of Bioorganic Chemistry PAS, Poznań, Poland

Catch me if you can: Unveiling circRNA functions through capturing RNA-protein interactions.

T15 **Robert Pasieka**, Adam Mickiewicz University in Poznań, Poznań, Poland

Exploring the interactions between lnc-ARRDC4-1, lnc-ADCYAP1-2, and DHX36: A novel axis in the translational and cellular pathways control

T16 **Jacek Nowak**, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

Discovery of the role of the PIWI-interacting protein Gtsf1 in the selective degradation of small RNAs in *Paramecium*

16:00 – 16:30 Coffee break

Afternoon Session II

16:30 - 18:15 Session on **RNA Modification**

Chair: **Tomasz Turowski**, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

T17 Monika Gaik, Małopolska Center of Biotechnology, Kraków, Poland

Deciphering Human Dihydrouridine Synthases: Structural Insights and Therapeutic Potential

T18 Marta Zimna, Adam Mickiewicz University in Poznań, Poznań, Poland

Pseudouridine – a new layer in plant microRNA biology

T19 Dawid Bielewicz, Adam Mickiewicz University in Poznań, Poznań, Poland

Unbiased identification of novel non-YTH putative m^{6}A readers from *Arabidopsis thaliana*

T20 Haider Ali, Małopolska Center of Biotechnology, Kraków, Poland

The m^{6}A modification of HIV-1 RNA is linked with a ribonucleoprotein MATR3-ELAVL1 complex

T21 Katarzyna Goljanek-Whysall, University of Galway, Galway, Ireland

Oxidised microRNAs – novel mechanism of muscle wasting?

T22 Elena Zemlyanskaya, Institute of Experimental Botany CAS, Prague, Czech Republic

RNA modification t^{6}A in plant development

T23 Piotr Gawroński, Warsaw University of Life Sciences, Warsaw, Poland

tRNA Sequencing Reveals a Blend of Ancestral and Acquired Post-Transcriptional Modifications in Chloroplast tRNAs

T24 Barbara Nawrot, Centre of Molecular and Macromolecular Studies PAS, Łódź, Poland

Sulfur- and selenium-modified uridines in the epitranscriptome of tRNA

18:15 – 19:30 Dinner

19:30 – 22:00 **Poster Session I** - wine, beer and snacks

Posters with odd numbers will be presented and discussed

L3

Complex metabolic pathways of endogenous and therapeutic mRNAs in vivo

Andrzej Dziembowski

International Institute of Molecular and Cell Biology in Warsaw

Mammalian mRNA turnover pathways were typically studied using model cell lines and rarely in vivo. In my talk, I will summarize our recent efforts to investigate the stability regulation of endogenous and therapeutic mRNA in more physiological setups. The analysis, which utilized transgenic mouse models and transcriptional analysis with tDirect RNA Sequencing, revealed high variability among cell types and tissues in pathways that either stabilize or lead to rapid degradation of mRNA. Additionally, the localization of the protein product can affect the metabolism of mRNA. For example, mRNA vaccines, which after intramuscular administration are mainly taken up by macrophages, are stabilized by TENT5-mediated cytoplasmic polyadenylation. This process is not active in model cell lines or in the liver. Concurrently, TENT5 proteins are localized at the endoplasmic reticulum, and only mRNAs encoding proteins targeted to membranes or secreted, such as antigens in the case of mRNA vaccines, are subject to such regulation.

10:15 - 12:00 Morning Session

RNA and Disease

T1

The role of structural elements within mutant mRNA of FMR1 gene containing expanded CGG repeats on the regulation of non-canonical translation of toxic polyglycine protein

Daria Niewiadomska; Izabela Broniarek; Anna Baud; Krzysztof Sobczak

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Uniwersytetu Poznanskiego 6, 61-614 Poznan, Poland

Repeat-associated non-AUG (RAN) translation of mutant *FMR1* mRNA containing expansions of CGG triplet repeats (rCGGexp) results in the production of a toxic polyglycine protein (FMRpolyG), which contributes to different fragile X premutation-associated conditions (FXPAC). The toxic rCGGexp is the template for both non-canonical translation of pathogenic FMRpolyG protein and canonical translation of natural product of the *FMR1*, FMRP protein. Due to the strong aggregation properties FMRpolyG is known to create intranuclear aggregates that lead to the disturbance of neurons and their death. The 5'UTR of *FMR1* mRNA folds into a thermodynamically stable secondary structure at the region of excessively expanded CGG repeats and constitutes a template for RAN translation initiated from near-cognate ACG or GUG start codons located upstream of the CGGs. Cis-regulatory elements, including sequence context and stable secondary structures within mRNA, can affect translation initiation and elongation. Here, we show that different nucleotide sequence contexts close to the near-cognate start codon of rCGGexp affect FMRpolyG synthesis. Moreover, the distance between the near-cognate start codon and downstream stable RNA structure considerably affects the efficiency of RAN translation initiation, which is positively correlated with the number of CGG repeats. In contrast, translation elongation is impaired as CGG repeats expand. We also show that native FMRpolyG containing a short polyglycine tract is synthesized efficiently but rapidly degraded by the proteasome; however, mutant protein containing long polyglycine sequence is not efficiently degraded by proteasome. Our results provide insight into the structural dependencies that regulate the translation of rCGGs and can be used in other repeat expansion disorders. We also show that the structure of *FMR1* mRNA is a potential therapeutic target in FXPAC.

T2

miR-199 regulates neuromuscular homeostasis during ageing

Maria Borja-Gonzalez[1]; Jose Casas Martinez[1]; Natalie Pollock[2]; Brian McDonagh[1]; Katarzyna Goljanek-Whysall[1,2]

[1] University of Galway, Galway, Ireland; [2] University of Liverpool, Liverpool, UK

Muscle wasting during ageing is irreversible and clinical management is limited and complex. The mechanisms underpinning age-related decline in muscle health are complex and include myofiber atrophy, defective muscle regeneration and deterioration of the neuromuscular interactions. microRNAs (miRs) are small non-coding RNAs that regulate expression of multiple genes post-transcriptionally. microRNAs have been implicated on regulation of muscle homeostasis during ageing and disease. We have demonstrated that among microRNAs dysregulated in muscle during ageing and ALS is miR-199a-5p. miR-199a-5p is predicted to regulate genes associated with mitochondrial dynamics and ER stress. Our data indicates the negative role of miR-199a-5p on myogenic differentiation and myotube size of C2C12 cells. Inhibition of miR-199a-5p in C2C12 also partially restored myotube atrophy resulting from Tunicamycin treatment of myotubes. Among genes regulated by miR-199a-5p overexpression or inhibition are CHOP, and GAP43. In vivo, overexpression of miR-199a-5p in muscle of adult mice led to decrease in muscle force as well as degeneration of the neuromuscular junction. Finally, inhibition of miR-199a in muscle of adult mice led to increase in muscle force, without an increase in muscle size. Together, these data indicate that inhibition of miR-199a may lead to preservation of muscle strength through regulation of neuromuscular interactions during ageing and potentially disease.

T3

C9orf72 mutation alters membrane protein expression in ALS and FTD

Urša Čerček[1], Jerneja Nimac[1,2], Vera Župunski[3], Boris Rogelj[1,3]

[1] Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia; [2] Graduate School of Biomedicine, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [3] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

Our study explores the genetic basis of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), with a particular emphasis on the widely occurring C9orf72 gene mutation. This mutation involves the expansion of a hexanucleotide repeat sequence - GGGGCC - transcribed in both sense (G4C2)n and antisense (C4G2)n directions, leading to the formation of nuclear RNA foci. Significant finding in our latest research is the discovery of an interaction between antisense C4G2 repeat RNA and phenylalanine-tRNA synthetase (FARS), resulting in reduced charging of phenylalanine-tRNA and consequent impact on the expression of proteins rich in phenylalanine content. Through bioinformatic analysis, we observed that the majority of proteins high in phenylalanine content localize to membranes, with the endoplasmic reticulum being notably prevalent. Utilizing techniques such as immunocytochemistry analysis and subcellular fractionation combined with western blots, we observed notable downregulation of these proteins in cells derived from C9orf72 patients. Our study highlights the significance of aminoacyl-tRNA synthetases and phenylalanine-rich proteins in C9orf72 ALS/FTD, suggesting their potential role in disease development. These innovative methods provide valuable tools for probing C9orf72 mutation-derived RNAs, paving the way for novel research avenues and therapeutic strategies.

References:

1 Črnigoj MM*, Čerček U* et al. Phenylalanine-tRNA aminoacylation is compromised by ALS/FTD-associated C9orf72 C4G2 repeat RNA. Nat. Commun. 2023 *Equal contribution

T4

Nuclear RNA decay gone wrong: how DIS3 mutations drive and then escape multiple myeloma

Tomasz Kuliński[1]; Olga Gewartowska[3,4]; Mélanie Mahé[5]; Karolina Kasztelan[1,2]; Janina Durys[2]; Anna Stroynowska-Czerwińska[6]; Marta Jedynak-Slyvka[7]; Ewelina P. Owczarek[1]; Debadeep Chaudhury[6]; Marcin Nowotny[7]; Aleksandra Pękowska[6]; Bertrand Séraphin[5]; and Andrzej Dziembowski[1,2,3]

[1] Laboratory of RNA Biology, International Institute of Molecular and Cell Biology, Warsaw, Poland; [2] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [3] Faculty of Biology, University of Warsaw, Warsaw, Poland; [4] Genome Engineering Facility, International Institute of Molecular and Cell Biology, Warsaw, Poland; [5] Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France; Centre National de Recherche Scientifique, Illkirch, France; Institut National de Santé et de Recherche Médicale, Illkirch, France; Université de Strasbourg, Illkirch, France; [6] Dioscuri Center for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [7] Laboratory of Protein Structure, International Institute of Molecular and Cell Biology, Warsaw, Poland

DIS3 serves as the ribonucleolytic core of the exosome complex, responsible for RNA degradation in the nucleus. DIS3 mutants exhibit severe mitotic defects, including sister chromatid separation failure. In yeast, these defects are linked to impaired processing of centromeric non-coding RNAs essential for kinetochore formation. Paradoxically, heterozygous point mutations in DIS3 inhibiting RNase activity of the enzyme are frequently found in multiple myeloma (MM), a blood cancer. Using knock-in mice with the clinical Dis3 G766R variant, we demonstrate how a ubiquitously expressed RNA-degrading enzyme drives oncogenesis specifically in the B-cell lineage. DIS3 mutant variant stalls on chromatin-bound substrates, leading to ectopic activation of AID, an essential component of immunoglobulin class switch recombination. Increased DNA double-strand breaks promote oncogenesis by facilitating translocations involving the immunoglobulin heavy chain (IGH) locus, key early drivers of myeloma. At the same time, DIS3-deficient cells accumulate centromeric non-coding RNA, particularly alpha-satellite transcripts. ChIP-seq analysis reveals minimal genome-wide chromatin changes, but significant centromeric deheterochromatinization, characterized by a shift from H3K9 tri-methylation to mono-methylation and increased H3K27 acetylation. These changes coincide with increased RNA polymerase II occupancy at centromeres and pericentromeric regions. Normally, centromeric heterochromatin is maintained by SUV39H1 methyltransferases, guided by alpha-satellite RNA, ensuring kinetochore integrity. DIS3-mutant cells exhibit proliferation defects, with blocks in the M phase and G1/S transition, resembling chaetocin-treated cells, which inhibit SUV39H1. Using CRISPR/Cas9 fluorescent labeling, we show that DIS3 mutations mislocalize SUV39H1 from centromeres, impairing heterochromatinization and cell division. Analysis of clinical samples revealed that, during MM progression, mutant DIS3 alleles that initially drive the disease are later eliminated. Accumulation of ncRNA species associated with DIS3 mutation is reversed upon loss of the mutant allele's heterozygosity, indicating that deregulated RNA impairs proliferation rather

than driving oncogenesis. Consistently, overexpression of wild-type DIS3 enhances the growth of DIS3-mutant myeloma cells, while CRISPR-mediated knockout of the mutant variant, followed by long-term co-culture, results in its counter-selection, mimicking its elimination observed during the course of the disease. DIS3 functions as a hit-and-run oncogene, initially driving MM but later shaping clonal evolution leading to its own removal.

T5

5' terminal nucleotide determines the immunogenicity of IVT RNAs

Magdalena Wolczyk*[1]; Jacek Szymanski*[1]; Ivan Trus*[1]; Zara Naz[1]; Tola Tame[1]; Agnieszka Bolembach[1]; Nila Roy Choudhury[1,2]; Karolina Kasztelan[1]; Juri Rappsilber[3]; Andrzej Dziembowski[1]; Gracjan Michlewski[1] (*-equal contribution)

[1] International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland; [2] MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Western General Hospital, Edinburgh, UK; [3] Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany

In vitro transcription (IVT) is a critical technology that has enabled significant advancements in mRNA therapeutics and driven major discoveries in RNA biology. IVT-produced RNAs typically begin with either a 5'-triphosphate guanosine (5'-pppG) or 5'-triphosphate adenosine (5'-pppA), both of which can serve as agonists triggering the RIG-I/type I interferon immune response. Although it is well-known that promoterless transcription during IVT can produce immunogenic double-stranded RNA (dsRNA), the specific role of initiating nucleosides in influencing this immunogenicity has remained unexplored. Our research demonstrates that RNAs synthesised through IVT starting with 5'-pppA exhibit significantly higher immunogenicity than their 5'-pppG counterparts. We detected elevated dsRNA levels generated by IVT RNAs beginning with 5'-pppA, which subsequently activated the RIG-I signalling pathway. Increased dsRNA levels were present in both short 5'-pppA RNAs and full-length mRNAs, including those used in COVID-19 vaccines. These findings identify a previously unrecognised factor contributing to IVT RNA immunogenicity, providing critical insights for advancing both academic research and therapeutic applications of IVT technology.

T6

Molecular characterisation of RNA-rich ensembles in Huntington's disease

Sandra Fienko; Iulia M. Nita; and Gillian P. Bates

Huntington's Disease Centre and Department of Neurodegenerative Disease, Queen Square Institute of Neurology, University College London, London, UK

Huntington's disease (HD) is caused by a CAG repeat expansion in exon 1 of the huntingtin gene. YAC128 HD mice are transgenic for a yeast artificial chromosome carrying the human HTT gene with an expanded CAG repeat. Our previous data indicate that human huntingtin pre-mRNA undergoes alternative processing to generate a small transcript comprising exon 1 and 5' intron 1 sequences (HTT1a) in the brains of YAC128 mice at two months of age. HTT1a encodes the highly pathogenic and aggregation-prone exon 1 HTT protein that exerts toxic effects on brain cells. Recently, we discovered that both fully processed human HTT and HTT1a mRNAs are retained in RNA nuclear clusters in the brains of YAC128 mice. These clusters may have pathogenic or protective consequences with potential therapeutical implications. To understand how early these HTT RNA clusters arise, mice at postnatal and embryonic stages were evaluated. Microscopic analysis revealed that HTT RNA clusters are detected at embryonic day 14.5, hinting at plausible early developmental abnormalities. Moreover, we determined that these clusters are mainly present in the neuronal but not glial cell population, implying a neuron-specific mechanism of accumulation. To interrogate the identity of these structures we performed combined RNAscope and immunofluorescence experiments. We observed that HTT RNA clusters are enriched in UAP56, a protein that couples mRNA splicing and export machineries and acts as an RNA remodeller. Simultaneously, these HTT RNA clusters are juxtaposed to PrP8, a central component of the spliceosome. We are currently pursuing high-throughput methods to interrogate the transcriptome and proteome of these clusters. To determine the dynamics of the HTT RNA clusters, we applied various pharmacological treatments in cultured hippocampal cells from YAC128 mice. Our experiments revealed that HTT RNA clusters can be dissolved upon application of nucleic acid intercalator agents that disrupt RNA gelation and transcription elongation inhibitors. We are now characterizing alternative processing and nuclear retention in the human tissue by interrogating post-mortem brain samples of individuals with juvenile HD onset. In-depth study of huntingtin RNA metabolism will facilitate understanding of HD pathogenesis and inform future preclinical trials.

T7

HTT loss-of-function contributes to RNA deregulation in developing Huntington's disease neurons

Emilia Kozłowska[1]; Agata Ciołak[1]; Grażyna Adamek[2]; Julia Szcześniak[1]; Agnieszka Fiszer[1]

[1] Department of Medical Biotechnology, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland; [2] Department of Neuronal Cell Biology, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

Huntington's disease (HD) is a neurodegenerative disorder caused by the expansion of CAG repeats in the *HTT* gene, which results in a long polyglutamine tract in the huntingtin protein (HTT). One of the earliest key molecular mechanisms underlying HD pathogenesis is transcriptional dysregulation, which is already present in the developing brain. In this study, we searched for networks of deregulated RNAs crucial for initial transcriptional changes in HD- and HTT-deficient neuronal cells. RNA-seq (including small RNAs) was used to analyze a set of isogenic human neural stem cells. The results were validated using additional methods, rescue experiments, and in the medium spiny neuron-like cells. We observed numerous changes in gene expression and substantial dysregulation of miRNA expression in HD and *HTT*-knockout (*HTT*-KO) cell lines. The overlapping set of genes upregulated in both HD and *HTT*-KO cells was enriched in genes associated with DNA binding and the regulation of transcription. We observed substantial upregulation of the following transcription factors: TWIST1, SIX1, TBX1, TBX15, MSX2, MEOX2 and FOXD1. Moreover, we identified miRNAs that were consistently deregulated in HD and *HTT*-KO cells, including miR-214, miR-199, and miR-9. These miRNAs may function in the network that regulates *TWIST1* and *HTT* expression via a regulatory feed-forward loop in HD. On the basis of overlapping changes in the mRNA and miRNA profiles of HD and *HTT*-KO cell lines, we propose that transcriptional deregulation in HD at early neuronal stages is largely caused by a deficiency of properly functioning HTT rather than a typical gain-of-function mechanism. Funding: National Science Centre (including 2021/41/B/NZ3/03803)

T8

Effective recognition of double-stranded RNA does not require activation of cellular inflammation.

Karolina Drazkowska[1]; Julia Cieslicka[1]; Michal Kitowicz[1]; Anna Pastucha[2]; Lukasz Markiewicz[3]; Wiktoria Szymanek[1]; Krzysztof Goryca[4]; Tomasz Kowalczyk[5]; Dominik Cysewski[5]; Andreas R. Bausch[2]; Pawel J. Sikorski[1]

[1] Laboratory of Epitranscriptomics, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland; [2] Center for Functional Protein Assemblies, Technical University of Munich, Munich, Germany; [3] Centre of New Technologies, University of Warsaw, Warsaw, Poland; [4] Genomics Core Facility, Centre of New Technologies, University of Warsaw, Warsaw, Poland; [5] Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland

Double-stranded RNA (dsRNA) in human cells originates from both endogenous and exogenous sources. Under optimal conditions, healthy human cells maintain low levels of dsRNA, which may arise from the RNA interference pathway, long non-coding RNAs, or transposable elements. However, in certain pathological conditions, dysregulation of RNA-processing machinery leads to excessive dsRNA accumulation. The primary exogenous source of dsRNA is viral infection, where dsRNA can serve as an intermediate in viral replication or constitute the viral genome itself. To counteract this potential threat, human cells have evolved sophisticated mechanisms for recognizing dsRNA and triggering an innate immune response, the first line of defense against viral infections. dsRNA recognition activates innate immune pathways, leading to inflammation and inhibition of cell growth. Here, we demonstrate that an effective dsRNA response can occur independently of inflammation. Notably, the pro-inflammatory RIG-I-like receptor (RLR) pathway and the cell growth-inhibitory OAS/RNase L and PKR pathways can function separately. We found that the 5’ end of dsRNA determines inflammatory signaling, while the RNA duplex structure activates the OAS/RNase L and PKR pathways. Surprisingly, three common human RNA epitranscriptomic modifications – N6-methyladenosine (m6A), 5-methylcytosine (m5C), and pseudouridine (Ψ) – had minimal impact on dsRNA immunogenicity. However, m6A selectively inhibited the OAS/RNase L pathway. These findings highlight the fine-tuned regulation of innate immunity, ensuring an appropriate cellular response to specific threats.

L4

From strings of nucleotides to collective behavior: “Lessons from Vibrio cholerae and its phages”

Kai Papenfort

University of Jena, Institute of Microbiology, Jena, Germany

Recently recognized to occur by the hundreds or thousands in the genomes of nearly all organisms, regulatory RNAs control complex biological functions ranging from embryonic development to bacterial virulence. In bacteria, small RNAs (sRNAs) constitute the best-studied class of non-coding regulators estimated to control up to 20% of all genes in a given organism. The sRNAs typically control gene expression by base-pairing with multiple trans-encoded target mRNAs rendering transcript stability and translation initiation. sRNA regulators are modular, versatile, and highly programmable, and therefore have gathered momentum as control devices in synthetic biology and biotechnology. In this presentation, I will highlight the mechanistic underpinnings of target recognition by bacterial sRNAs and link these regulatory events to the molecular functions that determine microbial physiology. Specifically, I will address how sRNAs control complex collective behaviors in bacteria, their effect on stress response systems, and the processes underlying RNA-mediated phage defense in bacteria.

14:15 -16:00 Afternoon Session I

Regulatory RNA

T9

RNA-triggered Cas12a3 cleaves tRNA tails to execute bacterial immunity

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Across all domains of life, tRNAs mediate transfer of genetic information from mRNAs to proteins on the ribosome. As their depletion arrests translation and, consequently, viral replication, tRNAs have long been recognized as key targets of innate immunity. Here, we describe adaptive immunity enacted by CRISPR-Cas12a3 effector nucleases that, upon recognition of complementary RNA, selectively cleave tRNAs, inducing growth arrest and countering phage infection in bacteria. Through direct-RNA sequencing, as well as biochemical and structural analyses, we demonstrate that activated Cas12a3 cleaves the conserved 5'-CCA-3' tail of diverse tRNAs by positioning them within its RuvC active site with the help of a distinct tRNA-loading domain. By mimicking tRNAs, we engineered custom substrates for Cas12a3, harnessing its specificity to expand multiplexed RNA detection of the established CRISPR-based diagnostic platforms. Overall, these findings reveal tRNA cleavage as a previously unrecognized CRISPR-based immune strategy and introduce new avenues for RNA-triggered manipulations within the CRISPR toolbox.

T10

Knockdown of *Smed ELAC2* in *Schmidtea mediterranea* decreases 5' tiRNA-Gly-GCC levels and reveals its regulatory role in regeneration

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The *Schmidtea mediterranea* flatworm is an excellent model for studying regeneration due to its remarkable ability to regrow the entire body, including the nervous system, from small tissue parts. RNA fragments derived from tRNA emerge as key regulatory molecules, alongside well-established regulators such as miRNAs and piRNAs, but their role in regeneration remains poorly understood. Ribonuclease Elac2 is highly conserved and involved in RNA maturation, with mutations in its gene leading to human Mendelian disease. In this study, we performed a knockdown of the *Smed ELAC2* gene in *S. mediterranea* and comprehensively investigated its impact on regeneration. Our results show that this process was significantly delayed, with impaired development of the central nervous system and photoreceptors. Notably, we observed a substantial decrease in the levels of 5' tiRNA-Gly-GCC, a tRNA half that has previously been shown to play an important role in fundamental processes across distant phyla. Functional *in vivo* analyses revealed that 5' tiRNA-Gly-GCC functions in a miRNA-like fashion, and enabled the identification of its target transcript. Our findings provide insight into the role of this small regulatory RNA in regeneration, presenting important mechanistic details. We propose that Elac2 is involved in the generation of 5' tiRNA-Gly-GCC, shedding new light on the biogenesis of tRNA fragments. These results not only enhance our understanding of the evolutionarily conserved differentiative processes but also have broader implications for elucidating disease mechanisms.

T11

The DEAD-box helicases DRH1, RH46 and RH40 remodel the secondary structure of miRNA precursors to regulate miRNA biogenesis in plants

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MicroRNAs (miRNAs) are short, single-stranded, non-coding RNAs that regulate gene expression at the post-transcriptional level. The process of miRNA production is complex and involves many proteins. Recently, DEAD-box helicases have become one of the most studied groups of proteins involved in miRNA biogenesis. The Arabidopsis genome contains 56 DEAD-box helicases. Of these, only DRH1, RH46 and RH40 contain WW domains, which have been identified by *in silico* analysis as a site for interaction with the CTD domain of RNA polymerase II (RNAPII). DRH1 is known to be involved in rRNA processing, nonsense-mediated decay (NMD) and mRNA export. However, the role of DRH1, RH46 and RH40 in miRNA biogenesis is unknown. During our research, we tested single, double and triple mutants of DRH1, RH46 and RH40 in Arabidopsis under standard (22°C) and modified (16°C) growth conditions. The phenotype of *drh1 rh46 rh40* plants was altered compared to wild-type plants when grown at 16°C. We observed that the absence of all studied helicases affected miRNA levels when grown under standard (22°C) growth conditions. Under the modified growth condition (16°C), this mutant showed enhanced changes in miRNA levels (mostly upregulated). Using FRET-FLIM analysis, we showed that DRH1 directly interacts with SERRATE (SE) and ARGONAUTE1 (AGO1), two important proteins in miRNA biogenesis pathways. In addition, DRH1 interacts with the CTD domain of RNAPII, consistent with *in silico* predictions. However, contrary to this prediction, we showed that the WW domain is not required for this interaction. We also investigated the secondary structure of selected pre-miRNAs in *drh1 rh46 rh40* using the targeted DMS-MaPseq technique. Our studies showed that the pre-miRNA secondary structure of a few pre-miRNAs is more condensed in the absence of the studied helicases. Taken together, our data suggest that DRH1, RH46 and RH40 influence miRNA biogenesis, possibly by shaping the structure of a subset of miRNA precursors. How the subset of precursors modified by the studied helicases is selected remains an open question. This work was supported by grants from the National Science Center UMO-2021/41/B/NZ1/03644 and UMO-2021/41/N/NZ1/03430.

T12

Tiny but mighty: Male-specific MpmiR11889 regulates proper sperm cell development and sexual reproduction efficiency in *Marchantia polymorpha*

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microRNAs (miRNAs) are master regulators of gene expression, fine-tuning developmental processes in the majority of eukaryotic organisms. In the early land plant *Marchantia polymorpha*, an emerging model for studying plant evolution and sexual reproduction, both evolutionarily conserved and liverwort-specific miRNAs orchestrate key developmental transitions. Notably, several miRNAs display distinct expression patterns in generative organs compared to vegetative thalli, suggesting critical roles in the development of reproductive structures. Among these, the male-specific MpmiR11889 is particularly intriguing. This ancient miRNA, conserved in both algae and liverworts, is uniquely expressed in sperm cells in Marchantia. Its only validated target is the mRNA encoding MpDUSP12, a dual-specificity phosphatase with deeply conserved functions across eukaryotes. In mammals, DUSP12 is involved in cell proliferation and differentiation, while in fungi it regulates sporogenesis. Importantly, DUSP12 contains a unique, evolutionarily conserved C-terminal domain harboring a zinc finger domain, suggesting its possible role in DNA binding. We show that in Marchantia, the MpmiR11889–MpDUSP12 regulatory module is indispensable for proper sperm development and reproductive success. Plants lacking MpmiR11889 (Δmpmir11889^{ge}) or overexpressing MpDUSP12 (MpDUSP12^{oe}) generate sperm with bead-like nuclei, elevated levels of a protamine-like protein (MpPRM) and altered flagellar structure of sperm cells. These chromatin abnormalities are confirmed by transcriptomic and proteomic profiling, which reveal upregulation of genes and proteins involved in chromatin architecture, DNA repair, and cellular growth. Functionally, sperm motility is severely compromised in the mutants, with disorganized and chaotic movement patterns. Reproductive fitness is also significantly impaired: crosses involving Δmpmir11889^{ge} or MpDUSP12^{oe} plants result in a substantial decrease in sporophyte formation efficiency (3–14%) compared to wild type. These findings establish the MpmiR11889–MpDUSP12 module as a key regulator of

chromatin remodeling and sperm cell functionality, highlighting ancient, conserved mechanisms underlying gamete differentiation. Funding: National Science Center (UMO-2020/39/B/NZ3/00539).

T13

Cytoplasmic synthesis of LINE-1 complementary ssDNA via self-primed reverse transcription

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LINE-1 retrotransposons are mobile genetic elements that mobilize through a copy-and-paste mechanism involving reverse transcription (RT) of LINE-1 mRNA directly into genomic DNA[1]. The genomic DNA liberated by LINE-1 endonuclease constitutes a primer for LINE-1 reverse transcriptase enzyme. LINE-1 sequences are scattered throughout human genome occupying nearly 17% of it. However, only few of these copies are full-length and capable of creating new genomic insertions. In fact, these are rare and form in either developing gametes or embryos when global DNA demethylation occurs to establish new organismal traits. In somatic cells, LINE-1s are strictly controlled and their activity restricted through DNA and histone methylation. Nevertheless, LINE-1s might be occasionally transcribed and translated in response to disease or ageing leading to adverse effects on their cellular hosts. We have pioneered analyses of LINE-1 mRNA 3' end qualities and discovered their pervasive uridylation i.e. addition of genomically non-templated U residues to the 3' ends of adenylated LINE-1 mRNA [2,3,4]. We demonstrated uridylation reduced LINE-1 retrotransposition deactivating its 3' end naturally involed in retrotransposition during the nuclear RT priming. LINE-1 is known to be source of extrachromosomal, cytoplasmic ssDNA that is sensed as a pattern stimulating cellular innate immunity and perpetuating sterile inflammation. Here, we created many different LINE-1 reporters to investigate how different pre-defined LINE-1 3' ends influence cytosolic RT priming. We show that uridylated LINE-1 mRNA can fold back to self-prime complementary ssDNA synthesis in the cytoplasm. This is supported *in cellulo* by immunofluorescence and *in vitro* by biochemical studies using *in vitro* transcribed RNAs and purified ORF2 LINE-1 reverse transcriptase. Furthermore, we investigated the effect of different 3' ends on ORF2 binding to LINE-1 mRNA 3' ends. Altogether we propose another layer of LINE-1 regulation by cellular modification of its 3' ends. These studies were supported by the National Science Center grant 2019/33/B/NZ1/02260 to ZW. 1 Warkocki (2023) FEBS Lett. pp. 380–406. PMID:36460901. 2 Warkocki, et al. (2018) Philos. Trans. R. Soc. Lond. B. Biol. Sci., 373(1762), p. 20180162. PMID:30397099. 3 Warkocki, et al. (2018) Cell, 174(6), pp. 1537-1548.e29. PMID:30122351. 4 Janecki, et al. (2024) Nucleic Acids Res. PMID:38197223.

T14

Catch me if you can: Unveiling circRNA functions through capturing RNA-protein interactions.

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RNA-binding proteins (RBPs) are indispensable players involved in RNA metabolism, and they can also be regulated by RNA. Circular RNAs (circRNAs) can modulate RBP function by acting as scaffolds, regulating their transport, or enhancing protein stability. The important roles for circRNA-protein interactions have been characterized in the nervous system for a handful of examples, and the functional roles of most endogenous circRNAs remain elusive. To investigate circRNA mechanisms of action, we employed high-throughput approaches to study native RNA-protein interactions. First, we applied XRNAX method to analyze RNA-protein interactomes in wild-type (WT) and dysmyelinated brains. Comparative analysis revealed enhanced nuclear translocation of MBNL1 protein in dysmyelination, coinciding with the significant reduction in *circMbnl1* expression. Interestingly, *circMbnl1* contains exons encoding the nuclear localization sequence (NLS). In line with the above, we showed that mRNA *Mbnl1* isoform with the exons carrying NLS is upregulated in dysmyelinated brain. That provides evidence that circRNA deregulation can influence the localization of the protein which is encoded by the same host gene as circRNA (Sztachera et al. 2024, *Cell Reports*). Second, we developed and optimized RNA pull-down protocol to isolate proteins bound to specific circRNAs. This method successfully enriched *Cdr1as* alongside its known partners such as miR-7 and AGO2. Subsequent LC-MS/MS analysis identified novel interactions between *Cdr1as* and other RBPs as well as synaptic proteins. These findings suggest *Cdr1as* may function as a synaptic protein scaffold, supporting its role in CNS physiology. Our study demonstrates that high-throughput RNA-protein interaction capture may help to elucidate circRNA functions in homeostasis sate and disease.

T15

Exploring the interactions between lnc-ARRDC4-1, lnc-ADCYAP1-2, and DHX36: A novel axis in the translational and cellular pathways control

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Long intergenic non-coding RNAs (lincRNAs) are RNA transcripts longer than 200 nucleotides, which usually exhibit tissue-specific expression. They can control nuclear architecture and transcription in the nucleus, as well as modulate mRNA stability and translation in the cytoplasm. However, although thousands of lincRNAs have been identified in human cells so far, knowledge of the majority of them remains rudimentary. My research focuses on the function of two lincRNAs: lnc-ARRDC4-1 and lnc-ADCYAP1-2. First, using TriplexAligner, we predicted the formation of triplex structures between double-stranded DNA and lnc-ARRDC4-1, indicating genomic regions that can be bound and further regulated by this lincRNA. These results are being confirmed by ChiRP-Seq. Next, we performed high-throughput sequencing of RNA isolated from cells expressing lnc-ARRDC4-1 and found that many genes predicted by TriplexAligner were indeed deregulated by lnc-ARRDC4-1 at the transcriptional level. Interestingly, among the targets is another lincRNA, lnc-ADCYAP1-2, whose expression increases along with lnc-ARRDC4-1. Mass spectrometry analysis revealed a significant overlap in changes in protein expression profiles between cells overexpressing lnc-ARRDC4-1 and those overexpressing lnc-ADCYAP1-2. Furthermore, antisense RNA purification coupled with mass spectrometry and RNA immunoprecipitation revealed that lnc-ADCYAP1-2 specifically interacts with the DHX36 helicase, which is involved in the resolution of G-quadruplexes within RNA structures and thus in translational regulation. Importantly, overexpression of lnc-ADCYAP1-2 results in a lower level of DHX36. Additionally, mRNAs identified in PAR-CLIP for DHX36 strongly correlate with the decrease in corresponding proteins in cells overexpressing lnc-ARRDC4-1 and lnc-ADCYAP1-2. Therefore, we suggest that upregulation of lnc-ADCYAP1-2, stimulated by lnc-ARRDC4-1, leads to inhibited DHX36 activity, thus stabilizing G-quadruplexes within mRNAs and ultimately reducing the translation of target proteins. In particular, the observed impact of this unique axis (lnc-ARRDC4-1: lnc-ADCYAP1-2: DHX36) extends to key cellular pathways, including replication, DNA repair, cell cycle regulation, cell adhesion, and cell migration. These findings were further supported by various functional assays, such as BrdU incorporation, comet assays, γH2AX foci quantification, XRCC1 foci disintegration kinetics, cell cycle analysis, migration assays, and adhesion assessments. In summary, these results highlight that lnc-ARRDC4-1 and lnc-

ADCYAP1-2 can regulate transcription and translation using different modes of action, with significant implications for cellular homeostasis.

T16

Discovery of the role of the PIWI-interacting protein Gtsf1 in the selective degradation of small RNAs in *Paramecium*

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Small RNAs in a wide range of organisms bind to PIWI proteins and guide them to complementary nascent transcripts, resulting in the recruitment of histone methyltransferase on chromatin and the repression of transposable elements and other repeats. In the ciliate *Paramecium*, during the development of the somatic macronucleus (MAC) from the germline micronucleus (MIC), that takes place at each sexual cycle, the genome is massively rearranged through the reproducible elimination of germline-specific sequences, including multi-copy transposons and thousands of single-copy internal eliminated sequences (IESs). Regulation of these programmed genome rearrangements is mediated by different types of non-coding RNA molecules: a special class of developmentally regulated small RNAs from the MIC (scnRNAs) and longer nascent transcripts produced both in the maternal MAC as well as in the new MAC, that are thought to act as pairing scaffolds for small RNA. 25-nt scnRNAs are produced from the entire germline genome during meiosis, bound by PIWI proteins and transported to the maternal somatic nucleus, where selection of scnRNAs corresponding to germline-specific sequences takes place. Selected scnRNAs are then translocated into the developing somatic MAC, where they recruit Polycomb Repressive Complex 2 (PRC2) and guide the deposition of histone H3 post-translational modifications (H3K9me3 and H3K27me3) onto transposable elements and IESs, triggering their elimination. Our studies provide important insights into the mechanism of scnRNA selection by identifying a *Paramecium* homolog of Gametocyte specific factor 1 (Gtsf1) as essential for the selective degradation of scnRNAs corresponding to retained MAC sequences. We provided the evidence that Gtsf1 is localized in the maternal somatic nucleus where it associates with the scnRNA-binding protein Ptiwi09. Moreover, we showed that in the absence of Gtsf1, H3K9me3 and H3K27me3 are not localized correctly in the new developing MAC and, in consequence, DNA elimination is impaired. Therefore, the scnRNA selection process occurring in the old MAC is crucial for the deposition of chromatin modification marks and genome rearrangements occurring in the new MAC. We propose a model in which Gtsf1 is required for the coordinated, ubiquitin-dependent degradation of Ptiwi09-scnRNA complexes that pair with homologous nascent RNA produced in the maternal MAC.

16:30 - 18:15 Afternoon Session II –

RNA Modification

T17

Deciphering Human Dihydrouridine Synthases: Structural Insights and Therapeutic Potential

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RNA modifications provide a sophisticated mechanism for regulating RNA structure and function. Among over 170 chemical alterations, dihydrouridine (D) is a prevalent modification found in tRNAs, mRNAs, snoRNAs, and rRNAs, playing a critical role in RNA biogenesis and protein translation. The unique non-aromatic structure of D enhances the flexibility of RNA molecules, particularly within tRNA D-loops and other loop regions. In humans, the conversion of uridine to dihydrouridine is mediated by four riboflavin-bound dihydrouridine synthases (DUS), each selectively targeting specific uridine sites across various RNA substrates. Despite its early discovery dihydrouridine remains a surprisingly underexplored tRNA modification. Increased expression levels of DUS enzymes are associates with pulmonary and glioma cancer types and have been linked to patients' poor prognosis. Moreover, dihydrouridylation may be linked to the onset of Alzheimer's disease. However, our knowledge about the exact molecular mechanism of DUS specificity and modification reaction remains limited. The crystal structures of bacterial DUS enzymes and cancer-related human DUS2 have been solved, exhibiting high structural similarity. Despite the predicted resemblance of DUS enzymes, each of the four human enzymes targets specific subsets of modifiable uridines in a variety of RNA targets, thus governing RNA substrate selectivity. Hence, resolving the structures of DUS-tRNA complexes is crucial for understanding how these enzymes recognize their tRNA substrates and catalyze the reduction of uridines at specific positions. Here, we combine macromolecular crystallography with complementary biochemical and biophysical analyses to broad our knowledge of dihydrouridine synthases (DUS) acting on human tRNA. All four purified enzymes interact with tRNA and modifies specific U-sites *in vitro*. Hence, we determined the impact of D incorporation on tRNAs stability. We solved the riboflavin bound apo-structures of human DUS1 and DUS2 catalytic domains at high resolution. Finally, we have conducted high-throughput screening of small molecule fragments library to identify lead compounds for binding to human DUS2 enzyme. Our studies lay a groundwork for designing molecular tools that can be further developed in diagnostics and clinical treatment

T18

Pseudouridine – a new layer in plant microRNA biology.

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MicroRNAs (miRNAs) are a pivotal component of gene expression regulation in animal and plant cells. Despite extensive research conducted over the past three decades following their discovery, numerous aspects of their biogenesis and action remain to be elucidated. The continuous development of novel tools and high-throughput methods in molecular biology has enabled the exploration of new dimensions of miRNA biology that were previously inaccessible. For example, in the past, technical challenges made it impossible to look for epitranscriptomic marks in small RNAs. The importance of RNA modifications in processes such as miRNA biogenesis is becoming increasingly evident. Our work is focused on pseudouridine (psi), sometimes described as “the fifth nucleotide” on account of its prevalence in many RNA sequences, mostly rRNAs and tRNAs. As pseudouridine has been now also found in plant miRNAs, we set out to find its role in miRNA biogenesis. We have found that the absence of psi-sites at specific positions in the miRNA/miRNA* duplex of ath-miR-159b might affect the efficiency of precursor processing. Following the evidence demonstrating that psi-sRNAs are transported between vegetative and sperm nuclei in pollen grains, with the involvement of PAUSED (PSD) transporter protein, we wanted to find out more about the role of pseudouridine in miRNA movement in the cell. For this, we’ve used a *psd-13 Arabidopsis thaliana* mutant, with a mutation within PAUSED encoding gene, and compared the pool of pseudouridilated miRNA pools between the nuclear and cytoplasmic fractions. In general, our results provide insight into a new level of understanding of plant miRNA biology.

T19

Unbiased identification of novel non-YTH putative m^{6}A readers from *Arabidopsis thaliana*

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Messenger RNA chemical modifications are recognized as crucial regulators of organismal growth, development, and reproduction. Among these modifications, methylation at the N^6 position of internal adenosines (m^{6}A) is the most abundant and widespread epitranscriptomic mark in eukaryotes. m^{6}A plays a critical role in various stages of mRNA metabolism, including transcription, maturation, export, stability, storage, and translation regulation. The molecular and cellular effects of m^{6}A are mediated by its interaction with "reader proteins," whose binding affinity for target mRNAs is influenced by the presence of this chemical modification. The most widely studied readers are those containing the YTH domain, a globular structure that forms an m^{6}A binding pocket. The high sequence conservation of these domains makes them easily identifiable across different genomes. In addition to the canonical YTH-domain readers, non-YTH readers have also been identified in mammals and more recently in *Arabidopsis thaliana*. However, their identification has been mostly serendipitous or relies on unbiased affinity capture techniques. To address this, we developed an affinity capture method using methylated and non-methylated RNA probes as baits to purify m6A readers from crude extracts of Arabidopsis seedlings. By performing label-free quantitative LC-MS/MS analyses on the affinity-enriched fractions, we identified 70 proteins specifically bound by the m^{6}A-RNA probes. Among these, we confirmed the capture of twelve out of the thirteen Arabidopsis YTH-domain proteins, which served as an internal control, validating the specificity of our assay for m^{6}A readers. We will present the methodology used for plant extracts, including the challenges and limitations encountered, as well as the significance of this approach in exploring m^{6}A-based regulatory pathways in plants. Additionally, we will discuss the list of putative non-YTH readers we identified in this study.

T20

The m^{6}A modification of HIV-1 RNA is linked with a ribonucleoprotein MATR3-ELAVL1 complex

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HIV-1 gene expression is intricately regulated by both viral and host factors to ensure efficient replication. Epitranscriptomic modifications of HIV-1 RNA introduce an additional layer of post-transcriptional regulation, influencing viral gene expression. One predominant epitranscriptomic modification is methylation at the N6-position of adenosine (m^{6}A), catalyzed by the "writer" protein complex METTL3/14/WTAP. This modification can be reversed by methylation "eraser" proteins ALKBH5 or FTO. The fate of m^{6}A-modified RNA is regulated by "reader" proteins, such as those from the YTH domain family. Several recent reports, including our own, indicate that m^{6}A modification on HIV-1 RNA enhances viral replication; however, the precise mechanistic basis of this regulation remains largely unclear. Previously, we identified that the nuclear matrix protein MATR3 plays a novel post-transcriptional role in the Rev-dependent nucleocytoplasmic export of viral transcripts— a critical step in the HIV-1 life cycle. Therefore, to elucidate MATR3 function, we explored its interactome using mass spectrometry and focused on ELAVL1 (HuR), a known m^{6}A "reader." Biochemical and microscopic analyses confirmed the interaction between MATR3 and ELAVL1, revealing that this interaction was RNA-dependent. Subsequent immunoprecipitation experiments in m^{6}A "writer" METTL3-depleted cells demonstrated that MATR3's interaction with both HIV-1 RNA and ELAVL1 was METTL3-dependent. Functional assays using CRISPR/Cas9 further established a role for ELAVL1 in HIV-1 reactivation from latency. Importantly, *in vitro* RNA-binding assays demonstrated that MATR3 preferentially binds m^{6}A-methylated RNA oligonucleotides, suggesting that MATR3 may function as a novel m^{6}A reader. To investigate this further, we are currently employing iCLIP and Cryo-EM to characterize MATR3's role in recognizing m^{6}A modifications. Findings from this study will enhance our understanding of m^{6}A modification and MATR3 in HIV-1 post-transcriptional regulation and may uncover new therapeutic targets for controlling or eradicating the virus.

T21

Oxidised microRNAs – novel mechanism of muscle wasting?

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MicroRNAs (miRs) control two thirds of the protein-coding transcriptome, and act as key regulators of development and disease. miR expression changes in multiple tissues during ageing and disease. There is growing evidence for a new gene expression paradigm, in which miR oxidation can lead to dysregulated interactions with target mRNAs. We hypothesised that miR oxidation represents a novel mechanism of epitranscriptomic reprogramming in tissue homeostasis, and oxi-miR accumulation during ageing or disease disrupts gene regulation, leading to tissue degeneration.. Given the extent of miR-mediated gene expression regulation, it is key to understand the potential and pitfalls of this phenomenon. We demonstrated that miRs are oxidised (oxi-miRs) during acute and chronic redox imbalance in muscle, using muscle samples from older people and cancer or COVID-19 patients, as well as mouse models of ageing, cachexia and critical illness. Using machine learning, we demonstrate microRNA features associated with increased likelihood of oxidation. We also identified several microRNAs consistently oxidised in muscle during pathological conditions. Next, we investigated the function of six oxidised miRs in vitro and in vivo. We demonstrated that oxidised miRs regulated different sets of genes than non-oxidised miRs and this results in phenotypic consequences. Moreover, miR oxidation appears to result in activation of cellular stress pathways. Finally, inhibition of oxi-miRs in vivo resulted in ameliorating loss of muscle size and strength in models of ageing and cachexia. Together, these data demonstrate fundamentals of miR oxidation, oxi-miR-mediated gene targeting and signalling during redox imbalance and further deciphers fundamental biological mechanisms of miR-mediated gene regulation, a phenomenon with enormous biological and biomedical impact and potential therapeutic implications for muscle loss and beyond.

T22

tRNA modification t^6A in plant development

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Among all RNA types, tRNA molecules possess an exceptionally high diversity of covalent modifications. However, a thorough understanding of the biological role of tRNA modifications, particularly in multicellular organisms, remains limited. We focus on exploring the effects of tRNA modification threonylcarbamoyladenosine (t^6A) in *Arabidopsis thaliana*. t^6A is one of the most ancient tRNA modifications that specifically marks A37 position of ANN-decoding tRNA molecules. t^6A is known to influence translation fidelity in eukaryotes. We characterize the components required for the t^6A formation in *A. thaliana* and reveal that major t^6A biosynthesis genes are vital for the earliest steps of plant morphogenesis. Furthermore, we show that in plants, t^6A is highly abundant in the form of a free compound, in contrast to other adenosine derivatives typically found in tRNA. Finally, by examining the subcellular localization of t^6A biosynthesis proteins, we present that the two sequential steps of t^6A biosynthesis are compartmentalized in Arabidopsis, with its first step occurring in plastids.

T23

tRNA Sequencing Reveals a Blend of Ancestral and Acquired Post-Transcriptional Modifications in Chloroplast tRNAs

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Chloroplasts in plant leaves are essential for protein synthesis, relying on tRNAs encoded by their own genome. Although post-transcriptional modifications are common in many non-coding RNAs, modifications in chloroplast tRNAs remain poorly characterized. In this study, we characterized post-transcriptional modifications in *Arabidopsis thaliana* chloroplast tRNAs using tRNA sequencing, liquid chromatography–mass spectrometry, and analysis of public datasets. Our results show that chloroplast tRNAs share modification patterns with bacterial systems—such as modifications at the anticodon-adjacent position and in the variable loop—while also exhibiting features typical of eukaryotic tRNAs that likely support their proper three-dimensional structure. Notably, we confirmed the presence of a unique 2-aminovaleramididine modification at the wobble position of tRNA-Ile^{CAU}, which is crucial for distinguishing isoleucine codons from methionine codons. These findings suggest that the chloroplast translation machinery, shaped by co-evolution with its eukaryotic host, incorporates both ancestral bacterial features and novel eukaryotic adaptations.

T24

Sulfur- and selenium-modified uridines in the epitranscriptome of tRNA

Barbara Nawrot

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tRNA molecules are among the most heavily and diversely modified RNA species. They undergo more than a hundred distinct chemical modifications that occur in different regions to stabilize the tRNA structure, ensure accurate codon-anticodon pairing, and fine-tune the overall efficiency and fidelity of protein synthesis. The most important tRNA modifications are found primarily in the wobble position of the anticodon loop (position 34). These modifications are of central importance as they enable the tRNA to pair with multiple codons, thereby expanding the decoding capacity and ensure that the genetic code is read accurately despite codon redundancy. These include sulfur- and selenium-modified uridines with different substituents in position 5 of the uracil ring. While 2-thiouridine modifications (R5S2U) occur in tRNA of all domains of life, the selenium-modified nucleosides (R5Se2U) are typically found in the tRNAs of certain bacteria. The modifications at the wobble position adjust the hydrogen-bonding potential of the uridine base and optimize it for precise recognition of the 3'-A/G ending synonymous codons in mRNA. In our studies, we have elucidated the function of R2S2U and R5Se2U in tRNA. We proposed a two-step linear biosynthetic pathway of R5Se2U-tRNA from R5S2U-tRNA via its S-geranylated derivative catalyzed by tRNA 2-selenouridine synthase (SelU) [1,2]. The pH-dependent potentiometric titrations allowed us to determine the pKa values of a series of modified S2Us and Se2Us and to confirm the hypothesis that mnm5S2U/ mnm5Se2U can adopt a zwitterionic form to efficiently bind the G moiety following a "novel wobble" mode [3-5]. The results of thermodynamic studies with R5S/Se2U-containing RNA duplexes suggest that the function of selenium in the wobble uridines of tRNA is to ensure the reading of the 3'-G-ending codons alongside the classical 3'-A-ending codons [6]. In addition, the redox properties of selenium make Se2U particularly interesting [7], as it tends to be more reactive to oxidation than S2U [8], which could play an antioxidant role under cellular stress conditions while aiding accurate decoding. The conversion between sulfur and selenium modifications can be influenced by environmental or redox conditions, adapting the translational performance to cellular needs. Acknowledgements: The author is grateful to the colleagues named in the cited papers from my laboratory and from the Institute of Organic Chemistry, Technical University of Lodz, Poland. This work was partly supported by the National Science Centre (NCN) in Poland and by statutory funds of TUL and CMMS PAS. [1] Sierant, M.; Leszczynska, G.; Sadowska, K.; Komar, P.; Radzikowska-Cieciura, E.; Sochacka, E.; Nawrot, B. FEBS Lett. 2018, 592(13), 2248-2258. [2] Szczupak, P.; Sierant, M.; Wielgus, E.; Radzikowska-Cieciura, E.; Kulik, K.; Krakowiak, A.; Kuwerska, P.; Leszczynska, G.; Nawrot, B. Cells 2022, 11, 1522. [3] Sochacka, E.; Lodyga-Chruscinska, E.; Pawlak, J.; Cypryk, M.; Bartos, P.; Ebenryter-Olbinska, K.; Leszczynska, G.; Nawrot, B. Nucleic Acids Res. 2017, 45, 4825. [4] Leszczynska, G.; Cypryk, M.; Gostynski, B.; Sadowska, K.; Herman, P.; Bujacz, G.; Lodyga-

Chruscinska, E.; Sochacka, E.; Nawrot, B. Int. J. Mol. Sci. 2020, 21, 2882. [5] Rozov, A.; Demeshkina, N,; Khusainov, I.; Westhof, E.; Yusupov, M.; Yusupova G. Nat Commun. 2016; 7:10457. [6] Leszczyńska, G.; Kulik, K.; Kuwerska, P.; Sochacka, E.; Nawrot, B.; 2025 in review [7] Kulik, K.; Sadowska, K.; Wielgus, E.; Pacholczyk-Sienicka, B.; Sochacka, E.; Nawrot, B. Int J Mol Sci. 2022 Jul 19;23(14):7973. [8] Sochacka, E.; Szczepanowski, RH.; Cypryk, M.; Sobczak, M.; Janicka, M.; Kraszewska, K.; Bartos, P.; Chwialkowska, A.; Nawrot, B. Nucleic Acids Res. 2015;43(5):2499-512.

Day 3, Thursday, June 26

08:00 - 09:00 Registration

09:00 - 9:45 Keynote Lecture

Chair: **Andrzej Dziembowski**, International Institute of Molecular and Cell Biology in Warsaw, Poland

L5 Bertrand Seraphin, Institute of Genetics, Molecular and Cellular Biology, Illkirch, France

Eukaryotic CCR4-NOT complexes: 1001 ways of regulating mRNA deadenylation and decay

9:45 - 10:15 Coffee break

Morning Session

10:15 - 12:00 Session on **Translation**

Chair: **Barbara Nawrot,** Centre of Molecular and Macromolecular Studies PAS, Łódź, Poland

T25 Haaris Safdari, University of Hamburg, Germany

The translation inhibitors kasugamycin, edeine and GE81112 target distinct steps during 30S initiation complex formation

T26 Ivan Sorokin, University of Groningen, Netherlands

Near-native mammalian cell-free protein synthesis system enabling mRNA translation in polysomes

T27 Khushboo Sharma, Charles University, Prague, Czech Republic

Deciphering Translational Regulation During the Cell Cycle Using Scarce Sample Polysome Profiling and Flow Cytometry

T28 Joanna Kufel, University of Warsaw, Poland

Non-canonical translation events in the yeast *Saccharomyces cerevisiae*

T29 **Marek Tchórzewski**, Maria Curie-Skłodowska University, Lublin, Poland

Ribosomal P-stalk: a harbinger-harmonizer of ribosome-factor interactions

T30 **Vladyslava Liudkovska**, IMol PAS, Warsaw, Poland

Translational Tuning of Sf1 Shapes the Splicing Landscape and Stem Cell Function

T31 **Katarzyna Knop**, Cancer Research UK – Scotland Institute, Glasgow, UK

Role of the RNA cap methylation in T-cell activation and differentiation

T32 **Witold Szaflarski**, Poznan University of Medical Sciences, Poznań, Poland

Mitoxantrone targets the nascent polypeptide exit tunnel to block translation, triggering the accumulation of 80S ribosome–rich stress granules

12:00 - 13:00 Lunch

13:00 – 13:45 Keynote Lecture

Chair: **Sebastian Glatt**, Małopolska Center of Biotechnology, Kraków

L6 **Elena Conti**, Max Planck Institute of Biochemistry, Martinsried, Germany

To degrade or not to degrade: molecular mechanisms of RNA homeostasis

13:45 - 14:15 Coffee break

Afternoon Session I

14:15 -16:00 Session on **RNA Structure** and **Viral RNAs**

Chair: **Elżbieta Kierzek**, Institute of Bioorganic Chemistry PAS, Poznań, Poland

T33 **Lukáš Pekárek**, Dresden University of Technology, Dresden, Germany

RNAs untangled: Shedding light onto complex structures of long RNAs

T34 **Palina Kot**, Justus Liebig University Giessen, Germany

Structural and Functional Insights into the Sub2-Yra1-Tho1 Complex in Nuclear mRNP Biogenesis

T35 **Mateusz Wilamowski**, Jagiellonian University, Kraków, Poland

Structural Insights into N4BP1 as an RNase within the mRNA Decapping Machinery

T36 **Angelika Andrzejewska-Romanowska**, Institute of Bioorganic Chemistry PAS, Poznań, Poland

Mapping the structural landscape of the RNA genome of the active Ty3 retrotransposon

T37 **Jakub Nowak**, Malopolska Centre of Biotechnology, Kraków, Poland

DyRNA Thermometry allows to monitor position specific structural changes in folded RNA

T38 **Tomasz Turowski**, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

Rate limiting steps in RNA synthesis during early SARS-CoV-2 infection

T39 **Ivan Trus**, International Institute of Molecular and Cell Biology in Warsaw, Poland

Unveiling Real-Time Innate Immune Responses with a Novel Fluorescent IFN-β Reporter Mouse

T40 **Martin Pospíšek**, Charles University, Prague, Czech Republic

Discovery of uncapped and 5'-polyadenylated mRNAs in poxviruses and yeast virus-like elements – comparison and similarities of both models.

16:00 - 16:30 Coffee break

Afternoon Session II

16:30 - 18:15 Session on **Computational RNA Biology**

Chair: **Marek Tchórzewski**, Maria Curie-Skłodowska University, Lublin, Poland

T41 Marcin Sajek, University of Colorado School of Medicine, Aurora, CO, USA

aa-tRNA-seq - nanopore based method for sequencing intact aminoacylated tRNAs

T42 Martyna Nowacka, Molecure SA, Warsaw, Poland

MoleRNA – discovering and designing small molecules targeting mRNA

T43 Bart Kris, Adam Mickiewicz University in Poznań, Poznań, Poland

Epigenetic control of transcriptional landscape during keratinocyte differentiation

T44 Michał Krzysztoń, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

Transcriptional activity during seed imbibition is conserved, robust and required for germination

T45 Monika Kwiatkowska, Institute of Bioorganic Chemistry PAS, Poznań, Poland

From Unknown to Unveiled: Revealing the Hidden Landscape of Zebrafish lncRNAs with CapTrap-CLS

T46 Marcin Tabaka, Institute of Physical Chemistry PAS, Warsaw, Poland

Inference of developmental processes and gene expression programs from single-cell multimodal data

T47 Natalia Ryczek, Adam Mickiewicz University, Poznań, Poland

Role of ELAVL1 in RNA:RNA duplex-mediated expression regulation of head-to-head overlapping protein-coding genes *INO80E* and *HIRIP3*

T48 Luiza Zuvanov, Free University of Berlin, Germany

FAM32A-mediated 3'-splice site selection controls germline and embryo development in *C. elegans*

18:15 – 19:30 Dinner

19:15 – 22:00 **Poster Session II** - wine, beer and snacks

Posters with even numbers will be presented and discussed

L5

Eukaryotic CCR4-NOT complexes: 1001 ways of regulating mRNA deadenylation and decay

Bertrand Seraphin

Institut de Génétique et de Biologie Moléculaire et cellulaire (IGBMC), Centre National de Recherche scientifique (CNRS) - Institut National de santé et de Recherche Médicale (Inserm) U964 - Université de Strasbourg, Illkirch, France

The eukaryotic multistep gene expression pathway, leading from nuclear encoded genes to protein production, involves minimally transcription, pre-mRNA processing, mRNA export from the nucleus, translation and RNA decay. Regulatory mechanisms and quality control processes impact each of these steps to allow cells to adapt to their environment and, for multicellular organisms, to permit harmonious development. Recent analyses have provided increasing evidence for the central contribution of mRNA decay to gene expression regulation. mRNA turnover is generally initiated by the gradual removal of the poly(A) tail by deadenylases until a dozen A residues is left. This first step is followed by cap cleavage that precedes 5'-3' exonucleolytic digestion of the mRNA body. Deadenylation is primarily mediated by the CCR4-NOT complex that appears as a main actor in the maintenance of proteostasis, from control of mRNA levels available for protein production to regulation of the translation mechanisms themselves.

Studies by us and others have provided some glimpses of the organization of the CCR4-NOT complex and its roles in controlling gene expression. Recent results revealed unanticipated mechanisms of control of mRNA deadenylation by the CCR4-NOT complex. In particular, a process involving its direct recruitment unfolded nascent polypeptides that contribute to protein quality control through selective mRNA deadenylation and decay will be presented.

10:15 - 12:00 Morning Session

Translation

T25

The translation inhibitors kasugamycin, edeine and GE81112 target distinct steps during 30S initiation complex formation

Haaris A. Safdari[1,5]; MartinoMorici[1,5]; Ana Sanchez-Castro[2,5]; Andrea Dallapè[1,3]; Helge Paternoga[1]; Anna Maria Giuliodori[4]; Attilio Fabbretti[4]; Pohl Milón[2]; Daniel N. Wilson[1]

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[5] These authors contributed equally: Haaris A. Safdari, Martino Morici, Ana Sanchez-Castro

During bacterial translation initiation, the 30S ribosomal subunit, initiation factors, and initiator tRNA define the reading frame of the mRNA. This process is inhibited by kasugamycin, edeine and GE81112, however, their mechanisms of action have not been fully elucidated. Here we present cryo-electron microscopy structures of 30S initiation intermediate complexes formed in the presence of kasugamycin, edeine and GE81112 at resolutions of 2.0-2.9 Å. The structures reveal that all three antibiotics bind within the E-site of the 30S and preclude 30S initiation complex formation. While kasugamycin and edeine affect early steps of 30S pre-initiation complex formation, GE81112 stalls pre-initiation complex formation at a further step by allowing start codon recognition, but impeding IF3 departure. Collectively, our work highlights how chemically distinct compounds binding at a conserved site on the 30S can interfere with translation initiation in a unique manner.

T26

Near-native mammalian cell-free protein synthesis system enabling mRNA translation in polysomes

Ivan Sorokin, Ekaterina Berlikova, Eugenia Panova, Albert Guskov, Zhanna Afonina, Sergei Dmitriev

Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Netherlands

Cell-free protein synthesis systems are essential tools for investigating mRNA translation and ribosome function. Current *in vitro* systems based on mammalian cell lysates produce proteins at low levels and, unlike living cells, fail to efficiently form and maintain polysomes. To address this limitation, we developed a comprehensive approach for preparing mammalian cell and organ lysates-based *in vitro* translation systems that preserve the activity of endogenous polysomes and enable their *de novo* assembly using synthetic mRNAs. Our approach optimizes key steps in cell lysis and mRNA translation, ensuring linear protein synthesis kinetics in polysomes for over three hours in a batch regimen. This system produces higher protein yields compared to existing alternatives and enables the rapid synthesis and reconstitution of multimeric protein complexes, along with the study of translation-inhibiting and -activating pathways. Overall, we provide a set of guiding principles that result in an efficient, cytoplasm-like cell-free system, making our approach a multi-tool for studying native translation events from both functional and structural perspectives, as well as for producing large quantities of proteins with mammalian-specific post-translational modifications.

T27

Deciphering Translational Regulation During the Cell Cycle Using Scarce Sample Polysome Profiling and Flow Cytometry

Khushboo Sharma; Kristina Roucova; Marharyta Ramanava; Martin Pospisek; and Tomas Masek

Laboratory of RNA Biochemistry, Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic

mRNA Translation is a highly regulated process and even more so during cell cycle transition wherein the activation or degradation of proteins mediate progression through the various phases. Genome-wide studies including proteomic and ribo-seq approaches show the importance of gene specific translation regulation along with global translation in cell cycle phases. However, the use of synchronisation obscures the accuracy of gene regulation patterns observed. There is also a lack of research data about the influence of mRNA features like UTR length, structure, and composition on it is translatability. Here, we coupled the well-established, high-sensitive polysome profiling method (Scarce sample polysome profiling; SSP-Profiling) with flow cytometry to obtain mildly fixed, unperturbed cells from different phases of cell cycle and evaluated their transcriptome and translatome. We confirm the distribution of light and heavy polysomal fractions with transcriptome but separation of non polysomal fraction along PC1 suggesting distinct variation in non-translated and translated mRNAs. Using differential gene expression, we preliminarily identify novel transcriptionally regulated genes in cell cycle. Furthermore, we characterise groups of translationally regulated gene and cluster them according to their translational trend in cell cycle. Our study establishes a new method for translation study in biologically limited samples with the possibility of coupled flow assisted sorting. It further delineates the translation landscape of cell cycle in unperturbed lymphoblastoid cells. This research was supported by the project OP JAC CZ.02.01.01/00/22_008/0004575 RNA for therapy, Co-Funded by the European Union and National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU and by The Czech Science Foundation (GACR, no. 22-27301S).

T28

Non-canonical translation events in the yeast *Saccharomyces cerevisiae*.

Maria Bozko[1]; Michal Swirski[1]; Håkon Tjeldnes[2]; Eivind Valen[2,3]; Pavel V. Baranov; Joanna Kufel[1]

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In addition to standard mechanisms, translation regulation also involves non-canonical events, including non-AUG initiation, stop-codon readthrough or programmed ribosomal frameshifting. These events may result in the the production of protein variants with different characteristics, such as subcellular localization or function, thus expanding the proteome. The identification of such rare non-canonical events, which may be more common than previously predicted, has recently been enabled by the availability of a growing number of Ribo-seq datasets. Analysis of large Ribo-seq dataset collections for yeast *Saccharomyces cerevisiae* using the recently developed RiboCrypt browser revealed several unexpected findings. First, we observed a characteristic signature of translation termination on standard stop codons within the ORFs. Although termination at AGA and AGG sense codons in human mitochondria or translation discontinuation induced by the nascent peptide N-terminal D/E-rich motifs in yeast have been reported, the mechanism using arbitrary sense codons as termination signals has not yet been characterised. The presence of specific termination signature enabled the detection of potential translation termination events in many protein-coding genes. Using standard biochemical assays, we have observed premature ribosome dissociation occurring in some cases with an efficiency as high as 40% and most often at positions near-cognate to stop codons. However, our preliminary results suggest that the mechanism responsible for this phenomenon does not depend on ribosome release factors but is to some extent linked to Ribosome-associated protein Quality Control (RQC) mechanism. We also observed efficient translation in intronic sequences in pre-mRNAs with retained introns exported to the cytoplasm. A stop codon in the intron usually causes degradation of such transcripts by NMD. However, we show that in some cases this situation leads to expression of truncated proteins, encoded either in the first or second exon. Both described non-canonical events may contribute to translatome diversity and regulate protein synthesis in different cellular or environmental conditions.

T29

Ribosomal P-stalk: a harbinger-harmonizer of ribosome-factor interactions

Kamil Filipek[1]; Sandra Blanchet[2]; Eliza Molestak[1]; Monika Zaciura[1]; Colin Chih-Chien Wu[3,10]; Patrycja Horbowicz-Drożdżal[1]; Przemysław Grela[1]; Mateusz Zalewski[4]; Sebastian Kmiecik[4]; Alan González-Ibarra[1]; Dawid Krokowski[1]; Przemysław Latoch[5]; Agata L. Starosta[5]; Mateusz Mołoń[6]; Yutian Shao[1]; Lidia Borkiewicz[1]; Barbara Michalec-Wawiórka[1]; Leszek Wawiórka[1]; Konrad Kubiński[7]; Katarzyna Socała[8]; Piotr Wlaź[8]; Kyle Cunningham[9]; Rachel Green[3]; Marina V. Rodnina[2] and Marek Tchórzewski[1]

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Protein synthesis is a highly regulated process, with the ribosome playing a central role in shaping the cellular proteome. This regulatory capacity relies on trans-acting factors and ribosomal elements, which frequently undergo post-translational modifications. In this context, the GTPase-associated center (GAC), particularly its primary functional element on the 60S subunit, the P-stalk, emerges as a key regulatory component. The GAC has dual functions: it engages with translational GTPases to support translation and interacts with the Gcn2 kinase, thereby linking ribosomes to the integrated stress response pathway. The P-stalk consists of three proteins, uL10, P1, and P2, which form a pentameric complex sharing a common C-terminal domain (CTD). We investigated the role of phosphorylation within the CTD of these proteins and found, that P-stalk proteins exist exclusively in a phosphorylated state. This ensures optimal translation during decoding and supports an effective Gcn2-dependent stress response. Molecular dynamics simulations suggest, that adding a phosphate group induces a transition from a collapsed globule to a coil-like structure, enhancing the P-stalk's ability to interact with various factors. Notably, unlike most ribosomal proteins, that are phosphorylated in an on/off manner, P-stalk proteins remain constitutively phosphorylated, maximizing their interactions with auxiliary factors.

T30

Translational Tuning of Sf1 Shapes the Splicing Landscape and Stem Cell Function

Vladyslava Liudkovska[1]; Ankita Kumari[1]; Martyna Ciołek[1]; Sandra Binias[2]; Anna M. Lenkiewicz[3]; Daniel Grygorowicz[1]; Anna Konturek-Cieśla[4,5]; Agata Szade[6]; Bartosz Wojtas[2]; David Bryder[4]; Krzysztof Szade[7]; and Maciej Cieśla[1]

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The transition of hematopoietic stem cells (HSCs) from quiescence to differentiation is essential for hematopoietic homeostasis. While transcriptional control of this process is well studied, the contribution of post-transcriptional mechanisms, particularly mRNA isoform diversity and translational regulation, remains poorly defined. We uncover a translationally regulated splicing program that directs HSC fate decisions and genomic stability. Specifically, we identify Sf1, a core branch point recognition factor, as a key regulatory node whose expression is tightly controlled via a conserved, structured 5' untranslated region (UTR). Loss of translational control of Sf1 through targeted deletion of the structural motif within UTR skews differentiation toward stem and erythroid states while impairing myeloid lineage commitment. Single-cell transcriptomics and splicing analyses reveal that Sf1 influences alternative splicing of 5' UTRs of transcripts governing hematopoietic differentiation and the DNA damage response. This affects translation efficiency and impacts genome integrity. Functionally, Sf1-deficient HSCs exhibit increased repopulation capacity but accumulate DNA damage, highlighting a trade-off between stemness and genomic stability. Our findings establish translational control of splicing factors as a critical post-transcriptional mechanism in hematopoiesis and suggest that targeting this axis may offer novel therapeutic strategies for hematologic disorders.

T31

Role of the RNA cap methylation in T-cell activation and differentiation

Katarzyna Knop; Alison Galloway; Carolina Gomez-Moreira; Victoria H. Cowling

Cancer Research UK – Scotland Institute, UK

T-cells are crucial for adaptive immunity, coordinating cell responses to pathogens by regulating both humoral and cellular responses. Upon interaction with pathogens, naïve T-cells undergo activation that requires a rapid increase of transcription, ribosome biogenesis and translation. The Cowling lab and others has shown that RNA cap methylation, in response to extra- and intracellular signals, affects transcription, RNA metabolism and translation. We used flow cytometry, mass spectrometry and RNA sequencing to investigate the role of RNA cap methylation in T-cell biology. We discovered that the RNA cap methyltransferases are differently regulated during the T-cell activation. Here we focused on the CMTR1 methyltransferase involved in methylation of the first transcribed nucleotide. That methylation is essential in marking RNA as "self" thus avoiding recognition by innate immune system and affects mRNA processing, stability and translation. Using conditional gene deletion mice, we found that CMTR1 is important for CD4 T cell activation, proliferation and differentiation. CMTR1 depletion influences mRNA and rRNA metabolism and translation, affecting the growth/proliferation, anti-inflammatory and cancer pathways in CD4 T cells. These findings indicate the hidden role of the RNA cap methylation in the modulation of the T-cell mediate immune and cancer response.

T32

Mitoxantrone targets the nascent polypeptide exit tunnel to block translation, triggering the accumulation of 80S ribosome–rich stress granules

Marta Leśniczak-Staszak[1,2]; Chih-Wei Chen[3,4]; Haaris A. Safdari[5]; Paulina Pietras[1]; Martino Morici[5]; Mateusz Sowiński[1]; Agnieszka Fedoruk-Wyszomirska[6]; Clifford Yerby[3,4]; Christiane Zorbas[7]; Ludivine Wacheul[7]; Max J. Berger[5]; Małgorzata Andrzejewska[1]; Jasbir Dalal[8]; Marcin Ruciński[1]; Ewelina Gowin[9,10]; Michał Nowicki[1]; Paul J. Anderson[11,12]; Eliza Wyszko[6]; Denis L.J. Lafontaine[7]; Shawn M. Lyons[8]; Pavel Ivanov[11,12]; Yuri Polikanov[3,4]; Daniel N. Wilson[5]; Witold Szaflarski[1]

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The anticancer drug mitoxantrone (MIT) was found to directly interact with the ribosome, specifically targeting the nascent polypeptide exit tunnel (NPET). Using X-ray crystallography on bacterial ribosomes and cryo-electron microscopy on human ribosomes, we determined that three MIT molecules bind within the NPET, physically obstructing the passage of the elongating polypeptide chain. These structural findings provided a mechanistic explanation for MIT's inhibition of protein biosynthesis, which we confirmed in both *in vitro* and *in vivo* systems. We also observed that MIT impairs ribosome biogenesis in the nucleolus, contributing to its anticancer activity. While preparing samples for structural studies, we noticed that MIT promotes ribosome aggregation. This observation prompted us to investigate MIT's potential to influence stress granule (SG) formation. Cellular experiments revealed that MIT induces SGs via a mechanism distinct from classical pathways dependent on eIF2α phosphorylation or mTOR inhibition. Strikingly, MIT triggered SG formation even under conditions of global translation inhibition, indicating that these granules assemble through a condensation-based process, likely facilitated by aggregated ribosomes. Further analysis showed that MIT-induced SGs contain a significantly higher abundance of large ribosomal subunits compared to sodium arsenite-induced SGs. This unique composition challenges the prevailing models of SG assembly and suggests an alternative pathway wherein SGs form through ribosome-driven liquid-liquid phase separation under conditions of severe cellular stress and damage. Together, our results identify MIT as a dual-acting

compound that not only disrupts translation and ribosome biogenesis but also promotes atypical SG formation through ribosome condensation.

L6

To degrade or not to degrade: molecular mechanisms of RNA homeostasis

Elena Conti

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In eukaryotes, the transcription of protein-coding genes is coupled to processing events (such as 5' capping, splicing and 3' polyA-tail addition) that modify the transcripts and coat them with proteins to form messenger ribonucleoprotein complexes (mRNPs). If all biogenesis steps occur correctly, the resulting mature mRNPs are transported through nuclear pore complexes by export factors. Failure in essentially any step of the biogenesis process can result in malformed mRNPs that are retained in the nucleus and eliminated by nuclear quality control pathways, primarily via the action of the RNA-degrading exosome. A large proportion of Pol II transcription indeed terminates before reaching the end of gene, resulting in degradation of the aborted transcripts. How do the quality control machineries recognize aberrant mRNAs/mRNPs and degrade them? Conversely, what are the features of mature, correctly packaged nuclear mRNPs that allows them to evade degradation? The talk will delve into our ongoing biochemical and structural studies, and discuss the current mechanistic understanding of RNA homeostasis.

14:15 -16:00 Afternoon Session I

RNA Structure and Viral RNAs

T33

RNAs untangled: Shedding light onto complex structures of long RNAs

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RNA is an intriguing molecule. Despite its relatively simple composition, RNA's functional versatility underscores the crucial role of RNA structure. Proper folding enables distant segments of the RNA molecule to come into close proximity, facilitating essential biological functions. This is particularly critical for long RNAs such as mRNAs, rRNAs, and lncRNAs, which can span over 1000 nucleotides. These long regulatory RNAs play diverse roles in gene regulation, chromatin organization, and post-transcriptional control. The function of these RNAs depends critically on their structure and ability to cooperatively interact with RNA-binding proteins, which often contain intrinsically disordered regions prone to condensation. When their structure or function is compromised, the consequences for the cell can be severe. This raises a fundamental challenge: how do living organisms ensure the robust and accurate folding of long regulatory RNAs? Do biological condensates help to shape RNA structures? And conversely, how do regulatory RNAs influence the formation of these condensates? In this project, we aim to understand how long RNA molecules fold into their complex structures and what the role of RNA-binding proteins is. We took lncRNA HOTAIR and YBX1 RNA-binding protein as a case study to shed some light on this folding enigma. By employing methods like single-molecule optical tweezers, fluorescence correlation spectroscopy, or confocal microscopy, we want to understand the key aspects of the dynamic folding process of complex RNAs in the context of biomolecular condensates.

T34

Structural and Functional Insights into the Sub2-Yra1-Tho1 Complex in Nuclear mRNP Biogenesis

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Processing, packaging into messenger ribonucleoprotein particles (mRNPs) and nuclear export of the mRNA are essential steps in eukaryotic gene expression, orchestrated by a network of RNA-binding proteins. In *Saccharomyces cerevisiae*, the conserved proteins Sub2, Yra1 and Tho1 play key roles in these processes. Sub2 is an ATP-dependent DEAD-box RNA helicase. Yra1 possesses RNA-annealing activity and serves as an adaptor for the conserved nuclear exporter Mex67-Mtr2. Both proteins are components of the TREX complex, which couples transcription to nuclear mRNA export. In contrast, the function of Tho1 in nuclear mRNP biogenesis remains unclear. However, all three proteins are nuclear mRNP components and form a complex. Despite their important roles, the structural and functional interplay between Sub2, Yra1 and Tho1 remains poorly understood. Here, we investigate the structural organization of the Sub2-Yra1-Tho1 complex by cryo-electron microscopy. The complex's heterogeneity necessitates its reconstitution with a defined RNA and purification using a sucrose density gradient. In addition, we explore the functional interplay of these three proteins. Specifically, Yra1 reduces the RNA-unwinding activity of Sub2. The structural and functional characteristics of the Sub2-Yra1-Tho1 complex are important for a detailed understanding of the molecular mechanism of nuclear mRNA export. Given the conservation of these proteins, an understanding of their function and regulation will also shed light on their roles in various diseases, including cancer and neurodegenerative disorders, positioning *S. cerevisiae* as a powerful model organism.

T35

Structural Insights into N4BP1 as an RNase within the mRNA Decapping Machinery

Mateusz Wilamowski[1]; Adrianna Graca[1,2]; Paweł Piłat[1,2]; Ankur Garg[3,4,5]; Kinga Rapacz[1,2]; Udo Heinemann[3,4]; Jolanta Jura[1]

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Exonuclease-mediated degradation of un-capped RNA is a fundamental mechanism in the primary immune response against exogenous RNA and tunes gene expression by cellular RNA surveillance. In eukaryotic cells, this process is tightly regulated and occurs within cytoplasmic granules known as processing bodies (P-bodies). Within P-bodies, mRNA decapping is initiated, followed by exonucleolytic degradation of the transcript dependent on action of XRN1. The decapping machinery is composed of a multiprotein complex centered around the scaffold protein EDC4, and includes EDC3, decapping enzymes DCP1A, DCP1B, and the mRNA cap hydrolase DCP2.

In this study, we aimed to uncover the function of human RNase N4BP1 within the context of macromolecular complexes involved in mRNA turnover. Through immunoprecipitation coupled with mass spectrometry, we identified N4BP1 as a nuclease associated with the mRNA decapping complex. Structural and functional characterization revealed that N4BP1 contains two KH (K-homology) domains and a PIN – nuclease domain, similar to Regnase1 RNase. The KH domains of N4BP1 are implicated in binding both RNA elements such as stem-loop motifs and possibly protein partners, stabilizing interaction with EDC4.

To explore the molecular architecture and functional domains of N4BP1, we analyzed its crystal structure (tandem KH domain), AlphaFold2 model and interaction interfaces, particularly focusing on regions critical for complex with EDC4. EDC4 serves as the structural core of the decapping complex, possesses a WD40 domain forming a stable seven-bladed β-propeller structure decorated with extended α-helices. Given the diversity of potential interaction partners, we implemented high-throughput cloning strategies derived from the MCSG structural genomics platform, resulting in the generation of over 200 expression vectors. These constructs are being used for comprehensive biochemical and structural analyses to define the role of N4BP1 both as an independent RNase and as a component of mRNA decapping machinery.

To study the N4BP1–EDC4 complex in detail, we are reconstituting the entire assembly *in vitro* using mammalian expression systems, specifically high-yield, suspension-adapted Expi293 and ExpiCHO cells. Following purification using affinity chromatography, N4BP1 complexes are subjected to electron microscopy, including negative-stain EM and cryo-EM, to solve their

structure and gain mechanistic insights into N4BP1's role in RNA metabolism and immune regulation.

T36

Mapping the structural landscape of the RNA genome of the active Ty3 retrotransposon

Andrzejewska-Romanowska Angelika; Gumna Julita; Tykwińska Ewa and Pachulska-Wieczorek Katarzyna

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Long terminal repeat (LTR)-retrotransposons are non-infectious mobile genetic elements widespread in eukaryotic genomes and crucial in their evolution. Retrotransposon RNA genomes (gRNA) serve as a template for protein synthesis and reverse transcription to a DNA copy, which can integrate into the host genome, thereby duplicating the element. The Ty3/Gypsy elements constitute an important and representative family of retrotransposons and are considered progenitors of retroviruses, such as HIV-1. Among them, the Ty3 retrotransposon, naturally occurring in yeast (*Saccharomyces cerevisiae*), is a widely used model to understand the biology of retroelements. Although both the sequence and structure of the RNA genome may carry the instructions necessary for replication, the structure of the Ty3 RNA genome has remained unknown so far. Here, we present the first secondary structure model of the entire RNA genome (5.2 kb) of the Ty3 retrotransposon replicating in yeast [1]. In brief, we employed the SHAPE-MaP (selective 2'-hydroxyl acylation analyzed by primer extension and mutational profiling) strategy to investigate the *in vivo* and cell-free structure of the Ty3 gRNA. Our study reveals the structural dynamics of the Ty3 gRNA and the stable, well-folded core, which is formed independently of the cellular environment. Based on the detailed map of the Ty3 gRNA structure, we characterized the structural context of *cis*-acting sequences involved in retrotransposition. We also identified a novel functional sequence as a potential initiator for Ty3 gRNA dimerization. Our data indicate that the dimer is maintained by direct interaction between short palindromic sequences at the 5' ends of the two Ty3 gRNAs, resembling the model characteristic for other retroelements like HIV-1 and Ty1. This work highlights a range of cell-dependent and -independent Ty3 gRNA structural changes, providing a solid foundation for studies on RNA structure-function relationships crucial for retroelement biology. [1] Andrzejewska-Romanowska et al., (2024). Mapping the structural landscape of the yeast Ty3 retrotransposon RNA genome. Nucleic Acids Research, 2024, 52(16), 9821-9837. This study was supported by the National Science Centre, Poland (project no 2020/39/B/NZ3/03020 to K.P.-W., 2021/41/N/NZ3/04060 to A.A.-R., and 2019/35/N/NZ1/01954 to J.G.).

T37

DyRNA Thermometry allows to monitor position specific structural changes in folded RNA

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RNA molecules not only contain linear sequence information, but they also fold into secondary structures and can attain complex three-dimensional shapes. Folded RNA molecules are dynamic and they can switch between different structural conformations. The low number of robust, simple and precise methodology to track these structural changes in RNA molecules limits our efforts to understand these dynamic processes. Here, we present DyRNA Thermometry, a fluorescence-based technique for monitoring RNA conformational stability in vitro. Leveraging the principle of the so called “temperature-related intensity changes” (TriC) effect, this fluorescence based method allows to measure the thermostability of individual position in RNA molecules. In this study, we tested the applicability of different fluorescent probes and benchmarked DyRNA Thermometry by monitoring the specific process of unfolding in small RNA hairpins. Furthermore, we show that the method is able to track domain-specific conformational changes in RNAs and sense ligand-induced conformation changes in different families of riboswitches. As the method is highly sensitive and requires little sample quantities it is highly suitable for high-throughput screening, needed in various biotechnological applications and the discovery pipelines for RNA-based therapeutics. The approach offers a versatile tool for structural studies of RNA, providing new insights into RNA stability and identifying optimal experimental conditions. Hence, DyRNA Thermometry's ability to detect subtle conformational shifts in RNA molecules provides a novel and complementary tool for RNA researchers in academia and industry.

T38

Rate limiting steps in RNA synthesis during early SARS-CoV-2 infection

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Coronaviruses (CoV) belong to a group of positive-strand RNA viruses, with a 30-kilobase-long, single-stranded RNA (ssRNA+) that serves as both mRNA and genome. Acting as mRNA, the viral genomic RNA directs the synthesis of non-structural proteins, including an RNA-dependent RNA polymerase (RdRP). This RdRP is responsible for synthesizing viral mRNAs and replicating the genome. During the synthesis of the (-) strand RNA, the RdRP shows discontinuous transcription, skipping large regions of the genome before resuming transcription. RdRP copies these (-) strand subgenomic RNA fragments (sgRNA-) into the viral mRNAs (sgRNA+), with their abundances dictating the stoichiometry of the numerous structural proteins. The mechanism of this „"jump"" and how the RdRP transitions between discontinuous and continuous transcription remains unclear. Deep RNA sequencing during the early stages of SARS-CoV-2 infection revealed novel mechanisms regulating RdRP transcription. Our analysis indicates low abundances of ssRNA(-) and sgRNA(-), with significant variability in the ratio between (+) and (-) strands across sgRNA types. In vitro transcription experiments recapitulating early infection stages showed impaired transcription efficiency of sgRNA(+) formation from sgRNA(-) templates. RNA secondary structure predictions suggest a robust structure at the common 3' end of ssRNA(-), potentially limiting accessibility for the polymerase. We hypothesize that the production of positive-strand sgRNA is a rate-limiting step in regulating the relative abundance of viral proteins in host cells. We developed a computational model for SARS-CoV-2 infection using a stochastic approach. This model integrates experimental data from early infection time points with reported protein stability, as well as transcription and translation kinetics. We propose novel mechanisms regulating CoV rate of RNA synthesis, including: (1) transient inhibition of translation initiation to resolve transcription-translation conflicts; (2) translation repression via protein-dependent and/or RNA-dependent mechanisms; (3) the rate-limiting role of RdRP; (4) a stochastic model for RdRP jumping; and (5) the inhibitory role of transcription and translation in viral RNA packaging. These insights help in understanding the key regulatory elements of CoV transcription.

T39

Unveiling Real-Time Innate Immune Responses with a Novel Fluorescent IFN-β Reporter Mouse

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Understanding the dynamics of innate immune activation is essential for unravelling host-pathogen interactions and developing effective immunotherapies. To enable real-time visualisation of IFN-β signalling, we engineered a genetically modified mouse model in which the IFN-β gene is replaced with the fluorescent reporter mKate2 (mKate2/IFN-β mouse). This system allows direct observation and quantification of both autocrine and paracrine IFN-β induction at the cellular and organismal levels. By minimising the feedback loop of interferon responses, our model captures early events in innate immune activation, providing a unique tool for studying the immunogenicity of nucleic acids. To demonstrate its utility, we transfected in vitro transcribed RNAs into bone marrow-derived macrophages (BMDMs) from mKate2/IFN-β mice. The IFN-β response was detectable within five hours and peaked at 20 hours post-transfection, with an enhanced sensitivity to double-stranded RNA compared to wild-type cells. In vivo, lipid nanoparticle (LNP)-mediated RNA delivery elicited a comparable response, further validating the model's physiological relevance. To investigate viral-induced IFN-β activation, we used an engineered influenza A virus (IAV) that co-expresses GFP alongside segment 3, as well as LNP-encapsulated mRNA encoding GFP. These experiments revealed that IFN-β activation varies depending on the source of GFP expression, with IAV-GFP infection eliciting a distinct immune response compared to LNP-encapsulated mRNA delivery. Differences in the fluorescent overlap between GFP and mKate2 signals suggest variations in mRNA processing, translation, or immune recognition of viral versus mRNA-expressed GFP, shedding light on the mechanisms driving IFN-β induction in response to different stimuli. In summary, our novel mKate2/IFN-β mouse model provides a powerful platform for dissecting innate immune activation in real time. Its applications extend beyond viral infections to broader studies on nucleic acid immunogenicity, making it a valuable tool for virology research and therapeutic development.

T40

Discovery of uncapped and 5’-polyadenylated mRNAs in poxviruses and yeast virus-like elements – comparison and similarities of both models.

Václav Vopálenský[1]; Michal Sýkora[1]; Zora Mělková[2]; Ivan Barvík[3]; Kristýna Ľalíková[1]; Kamila Horáčková[1] and Martin Pospíšek[1]

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Cytoplasmic double-stranded DNA virus-like elements (VLEs) are present in yeast species across nine genera. VLE archetypes are linear DNA elements pGKL1/2 from the yeast *Kluyveromyces lactis* . They exhibit unique features, including cytoplasmic localization, terminally linked proteins, and compact genomes carrying altogether 15 genes coding, besides others, for a killer toxin, DNA and RNA polymerases, a helicase, and a capping enzyme. Functions of many pGKL1/2 genes remain unclear. However, proteins that are essential for cytoplasmic transcription of pGKL1/2-encoded genes have been characterised in more detail and demonstrated substantial similarity to their functional counterparts in vaccinia virus (VACV). We found recently that although VLEs encode their putative capping enzyme, most of their mRNAs are uncapped and lack 3'-polyadenylation. Unexpectedly, many VLE mRNAs contain short 5' poly(A) sequences which are not complementary to their genomic DNA. Considering similarities in transcriptional apparatuses of VLEs and poxviruses, we further analysed also poxviral mRNAs. VACV, a prototypical poxvirus that was crucial for the smallpox eradication, played a key role in discovering the eukaryotic 5' mRNA cap and mechanism of its synthesis. Curiously, our analysis of individual VACV mRNAs revealed that postreplicative VACV transcripts in addition to presence of non-templated 5' poly(A) leaders lack a 5' cap. We found that cap occurrence in viral mRNAs decreases from early to late viral transcripts and similarly as in VLE mRNAs correlates inversely with increasing length of the 5' non-templated poly(A) leaders. Furthermore, the sequence of transcriptional initiator (INR) influences capping frequency and poly(A) leader formation by similar mechanism in yeast VLEs and VACV. Our findings also suggest that 5' mRNA polyadenylation may induce its cap-independent protein synthesis via an unknown mechanism. Our results indicate that switch in synthesis of capped to non-capped but 5’-polyadenylated viral mRNAs may facilitate transition from cap-dependent to cap-independent synthesis of VACV proteins and thus my help the virus to take over the host translation machinery in the course of the viral infection. Acknowledgement The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU and Project OP JAC CZ.02.01.01/00/22_008/0004575 RNA for therapy.

16:30 - 18:15 Afternoon Session II

Computational RNA Biology

T41

aa-tRNA-seq - nanopore based method for sequencing intact aminoacylated tRNAs

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Transfer RNAs (tRNAs) are the fundamental adaptors of translation, linking mRNA decoding and polypeptide synthesis. They are decorated during biogenesis with a variety of modifications that modulate their stability, aminoacylation, and decoding potential during translation. The complex landscape of tRNA modification presents significant analysis challenges and to date no single approach enables the simultaneous measurement of modifications and charging – important but disparate chemical properties of individual, mature tRNA molecules. We developed a new, integrated approach to analyze the sequence, modification, and aminoacylation state of tRNA molecules in a high throughput nanopore direct RNA sequencing experiment, leveraging a chemical ligation with phosphorimidazole-activated adaptors that embeds the charged amino acid in an adapted tRNA molecule. During nanopore sequencing, the embedded amino acid generates unique distortions in ionic current and translocation speed, enabling application of machine learning approaches to classify charging status and amino acid identity. Utilized machine learning models shown very high performance in distinguishing charged and uncharged tRNAs with F1 score > 0.9 and correlates well with experimental data. Specific applications of the method indicate it will be broadly useful for examining relationships and dependencies between tRNA sequence, modification, and aminoacylation in both physiological and pathological conditions. It is especially important taking into account that 56 human diseases are caused by mutations in aminoacyl-tRNA synthetases and in most of the cases molecular mechanism remains unknown.

T42

MoleRNA – discovering and designing small molecules targeting mRNA

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Molecure is developing a unique RNA platform to discover small-molecule compounds that interact directly with the mRNA of disease-related proteins. This approach is an alternative to conventional therapeutic strategies that involve direct targeting of dysfunctional proteins and offers access to potentially thousands of new therapeutic targets that were previously considered ‘undruggable’. We utilize the fact that mRNAs adopt specific three-dimensional (3D) structures, which are integral and essential for controlling mRNA life cycle and biological functions, specifically translation. Using computational methods and experimental approaches we identify stable and functional fragments of mRNA encoded by genes that are clinically significant in oncology and inflammation. The predicted structures of these fragments are then confirmed at single-nucleotide resolution using chemical probing and structural biology techniques, such as Cryo-EM. In one of our mRNA projects, we target the specific structure in 5’UTR of mRNA1, whose functional significance was assessed using antisense oligonucleotides (LNA) and RNA-protein pulldown experiments. Subsequently, in our pipeline, DNA-encoded library screening (DEL) was applied to identify new molecules with therapeutic potential. Hit molecules selected from billions of small compounds were tested using biophysical and cell biology methods ultimately identifying Hit molecules that dose-dependently reduced the level of protein encoded by mRNA1. In our second mRNA project, we utilized a Virtual Screening-based approach to identify compounds, that are most likely to bind to the 3D RNA structure localized in the 3’UTR of mRNA2 and obtained from computational modeling supported with chemical probing data. A subset of hit molecules identified through this process was selected for biological evaluation. Hits exhibiting a significant reduction in protein2 level entered the Med-Chem campaign to develop drug-like molecules relevant to suppressing oncogenesis-related processes. In parallel, we are constantly expanding our screening approaches. For example, 19F-NMR Fragment-Based Drug Discovery is currently applied to identify new molecules with therapeutic potential that interact with functionally validated mRNA regions and modulate the level of target proteins. Molecure’s intention is to provide scientists and the biopharmaceutical industry with a platform for

identifying starting compounds, confirming that mRNA can be a therapeutic target for small molecules.

T43

Epigenetic control of transcriptional landscape during keratinocyte differentiation

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Mammalian epidermis is a stratified, self-renewing epithelium consisting of several layers of keratinocytes crucial for survival. As keratinocyte undergo differentiation they move upwards, loose their proliferative capacity while upregulating genes involved in epidermal barrier formation to finally undergo skin-specific version of programmed cell death: corneoptosis, which is indispensable for the formation of fully functional skin. Those dramatic changes are driven by transcriptional program tightly controlled by several epigenetic pathways and affected by remarkable changes in 3D genome architecture. Our results show how changes in nuclear compartments set by repressive Polycomb complexes PRC1 and PRC2, as well as nuclear lamina and nucleoli contribute to establishment of the transcriptional profiles of distinct subpopulations of keratinocytes. As the differentiation progresses the amount of chromatin bound to nucleoli and nuclear lamina increases, similarly to the global level of Polycomb repression. Those changes, besides directly affecting transcription, work also in more nuanced way by changing the chromatin folding and rewiring of promoter-enhancer interactions. Intriguingly, most keratinocyte-specific structural genes are located within three genomic loci: EDC, Ker I and Ker II. EDC spanning 3Mbp is inflicted in several diseases including psoriasis and atopic dermatitis. We show that despite high activity part of the EDC is associated with nuclear lamina which seem to fine tune its gene expression together with changes in the activity of PRC1/2. To sum up our results coming from set of genomic experiments provide novel insight into the role of the PRC1/2 complexes as well as changes in 3D nuclear architecture in the control of gene expression during establishment of epidermal barrier.

T44

Transcriptional activity during seed imbibition is conserved, robust and required for germination

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Germination begins with the reactivation of metabolism in desiccated seeds during water uptake. However, Pol II transcription is considered not required for early germination, as seeds initially rely on stored mRNAs produced during maturation. Through phenotypical and transcriptomic analyses, we discovered that efficient blocking of Pol II transcription completely halts germination in Arabidopsis seeds. By employing new mRNA labelling, we demonstrated that Pol II activity is initiated early and is widespread, following gene expression patterns established during seed drying. This early transcriptional programme is robust across diverse imbibition conditions and in seeds matured at varying temperatures, and it is conserved in *Brassica napus*. Translation inhibition suggests that early transcription occurs in two phases, the first of which is independent of factors generated de novo during imbibition. The pattern of the first transcriptional wave is partly explained by Pol II binding in the chromatin of dry seeds. Only after successfully completing this initial phase can seeds activate the genes necessary for germination. Our results redefine the role of transcription in seed germination and illuminate a conserved pattern of early transcription required for this process.

T45

From Unknown to Unveiled: Revealing the Hidden Landscape of Zebrafish lncRNAs with CapTrap-CLS

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Vertebrate genomes encode thousands of long noncoding RNAs (lncRNAs)—transcripts exceeding 200 nucleotides with limited protein-coding potential. Despite accumulating evidence of their roles in essential biological processes, over 97% of lncRNAs remain functionally uncharacterized. The use of animal models can advance our understanding of lncRNA functions; however, the success of such studies depends heavily on the quality of genome annotations. While zebrafish (*Danio rerio*) has emerged as a valuable vertebrate model for lncRNA research, its genome annotation lags far behind that of humans or mice, significantly limiting its utility. To address this gap, we developed the CapTrap-CLS protocol to generate a complete and accurate catalog of lncRNA genes in the zebrafish genome. CapTrap-seq is a full-length library preparation method that excels in selectively enriching for 5' and 3'-complete transcripts, enabling more precise annotation of transcription start sites (TSSs) for biologically relevant genes while simultaneously and efficiently reducing rRNA contamination (<0.01%). To enhance the detection of lowly expressed lncRNAs, CapTrap-seq was integrated with the CLS approach—a targeted RNA sequencing method combining RNA capture with long-read Nanopore sequencing. This strategy efficiently enriched lncRNAs, increasing their representation in post-capture libraries. Additionally, targeted RNA sequencing of human-mouse-zebrafish syntenic regions not only identified novel transcript isoforms for potentially functional lncRNAs but also significantly improved the detection of novel genes in intergenic regions, highlighting the utility of positional conservation for lncRNA gene discovery. Altogether, the application of the CapTrap-CLS method resulted in the first comprehensive full-length lncRNA dataset for zebrafish, identifying 12,000 novel lncRNA genes and thereby expanding the catalog of lncRNA loci fourfold while doubling the average number of transcript isoforms per gene compared to the Ensembl database. This improved zebrafish genome annotation provides a strong foundation for advancing the use of zebrafish as a model organism for studying lncRNA functions and is expected to facilitate homology-based lncRNA gene prediction across species, including humans.

T46

Inference of developmental processes and gene expression programs from single-cell multimodal data.

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The recent expansion of single-cell sequencing technologies has enabled simultaneous genome-wide measurements of multiple modalities in the same single cell. The potential to jointly profile such modalities as gene expression, chromatin accessibility, proteins, or multiple histone modifications at single-cell resolution represents a compelling opportunity to study biological processes at multiple layers of gene regulation. Analysis of single-cell multimodal profiling experiments poses significant computational challenges because each single-cell data modality is high-dimensional. The number of measured features spans from hundreds in the case of protein epitopes to hundreds of thousands for chromatin-accessible sites. The multiple modalities profiled correspond to consecutive stages of gene expression, from its regulation by modifying chromatin architecture and engaging transcription-initiation proteins to the synthesis of mRNA and protein molecules. Thus, all modalities need to be modeled simultaneously to analyze and visualize multimodal data. I will present recently developed experimental multimodal profiling technologies and machine learning methods in our laboratory for exploration of gene expression programs and their regulatory mechanisms.

T47

Role of ELAVL1 in RNA:RNA duplex-mediated expression regulation of head-to-head overlapping protein-coding genes *INO80E* and *HIRIP3*

Natalia Ryczek; Aneta Łyś; Elżbieta Wanowska; Joanna Kozłowska-Masłoń; Izabela Makałowska

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The presence of overlapping protein-coding genes in eukaryotic genomes has been recognized for decades, yet their role in gene expression regulation remains unclear, with studies yielding inconsistent results. To explain the mechanisms governing the expression of head-to-head overlapping genes, *INO80E* and *HIRIP3* gene pair were investigated. Through a series of experiments, we demonstrated that this gene pair expression is highly dependent on sense/antisense interactions. The overlapping transcripts form an RNA:RNA duplex, which stabilizes the involved mRNAs. Our study reveals that ELAVL1, a well-known RNA-binding protein, plays a crucial role in the post-transcriptional regulation of this gene pair via an RNA duplex-mediated mechanism. We performed experiments, including CLIP-qPCR, which indicated that the ELAVL1 protein selectively binds to these duplexes, modulating their processing and degradation, thereby fine-tuning the expression levels of both genes. In subsequent experiments, we demonstrate that loss of the RNA:RNA duplex leads to decreased chromatin accessibility and increased transcriptional interference, disrupting the coordinated expression of *INO80E* and *HIRIP3*. Altogether, these findings provide new insights into the molecular mechanisms underlying overlapping gene regulation. Our study highlights the importance of sense/antisense interactions and RNA structure in maintaining transcriptional balance, challenging the traditional view that overlapping genes necessarily interfere with each other's expression. Instead, our results suggest that under specific conditions, overlapping transcription can facilitate gene expression by stabilizing transcripts and maintaining chromatin accessibility.

T48

FAM32A-mediated 3’-splice site selection controls germline and embryo development in *C. elegans*.

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Alternative splicing (AS) is a potent and widely used source of transcriptome diversity. Tandem alternative 3'-splice site (TASS) is a subtype of AS ubiquitously found in the eukaryotic lineage. The choice of proximal (PS) or distal 3’-splice site (DS) was shown to be regulated in a tissue-specific manner in mammals (Bradley, 2012). In the nematode *Caenorhabditis elegans*, this regulation is stronger and monodirectional, where somatic cells prefer DS and gonads opt for PS usage (Ragle, 2015). However, the biological relevance of this regulation remains unclear. To investigate this further, we developed a de novo Pipeline for Identifying RNA Alternatives (PIRA) that haven’t been previously detected. During the *C. elegans* life cycle, our pipeline detected 308 new regulated TASS events. Developmentally regulated events were found preferentially towards the 3’-end of the transcripts and, in the vast majority, are non-disruptive, including 6nt or 9nt. Further, based on our mechanistic work about the human spliceosomal C* complex (Dybkov & Preussner, 2023), we also decided to address the functionality of TASS selection. To this end, we created a *C. elegans* mutant strain of the FAM32A human homolog (K01G5.8), a potent activator of human PS usage. RNA-Seq analysis of our mutant worms lacking the conserved ceFAM32A C-terminus revealed primarily changes in TASS selection compared to control samples: >100 and >400 differentially spliced events were detected in L3 and adult worms, respectively. Phenotypically, 15% larval arrestment and 7% sterility were observed, along with a 60% reduction in egg laying. At 25°C, 50% of worms showed no hatched eggs. Interestingly, publicly available RNA-seq data analysis shows that TASS selection is heavily regulated during embryogenesis. We observed that embryos predominantly use PS until the 8-cell stage, whereas later stages show an abrupt change towards DS usage. This shift is concomitant with the gastrulation onset and oviposition. Together, our findings suggest a key function of ceFAM32A in germline and egg development. In this way, our work provides evidence for a conserved regulator of TASS usage across species. Understanding how this mechanism operates may help elucidate the role of alternative splicing in cell fate determination and transcriptome plasticity in metazoans.

Day 4, Friday, June 27

09:00 - 9:45 Keynote Lecture

Chair: **Magda Konarska**, IMol PAS, Warsaw, Poland

L7 **Reinhard Lührmann,** Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

Structural insights into the cascade of snRNP remodeling steps leading to the formation of a catalytically activated spliceosome

9:45 - 10:15 Coffee break

Morning Session

10:15 - 11:30 Session I on **Transcription and mRNA Processing**

Chair: **Magdalena Masłoń**, Małopolska Center of Biotechnology, Kraków, Poland

T49 **Michał Rażew**, European Molecular Biology Laboratory, Grenoble, France

Structural basis of the Integrator complex assembly and association with transcription factors

T50 **Magda Kopczyńska**, Adam Mickiewicz University, Poznań, Poland

SETD2 methyltransferase activity aids gene definition by promoting correct transcription initiation and termination

T51 **Jan Mikołajczyk**, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

Comprehensive mapping of active transcription by human RNA polymerase III reveals a rate-limiting role for transcription termination

T52 **Bogdan Cichocki,** IMol PAS, Warsaw, Poland

The by-product of spliceosomal first-step catalysis protonates adjacent catalytic triplex residues, promoting transition to the second step

T53 Ankita Kumari, IMol PAS, Warsaw, Poland

Implications of spliceosome control during the malignant hematopoietic activation

T54 Mateusz Dróżdż, Free University of Berlin, Germany

Immediate early splicing after T cell activation is controlled by temporal phosphorylation of hnRNPC2

11:30 - 12:00 Coffee break

Afternoon Session

12:00 – 13:15 Session II on **Transcription and mRNA Processing**

Chair: **Zbigniew Warkocki**, Institute of Bioorganic Chemistry PAS, Poznań, Poland

T55 Dominique Gagliardi, University of Strasbourg, France

Antagonistic effects of uridylation and deadenylation shape poly(A) tail profiles in Arabidopsis

T56 Maciej Śmiałek, University of Basel, Switzerland

CFIm-Complex-Driven Alternative Polyadenylation Controls mRNA Length, Stability, Localization and Protein output in colon and other cancers

T57 Natalia Gumińska, International Institute of Molecular and Cell Biology in Warsaw, Poland

Tracking poly(A) tail diversity with Ninetails: mixed tails from cells to mRNA therapies

T58 Mateusz Bajczyk, Adam Mickiewicz University in Poznań, Poznań, Poland

The role of PCF11-similar proteins in alternative polyadenylation and flower development in *Arabidopsis thaliana*

T59 Agnieszka Czarnocka-Cieciura, International Institute of Molecular and Cell Biology in Warsaw, Poland

Decoding mRNA Fate: Poly(A) Tail Dynamics and the Role of Puf Proteins in Deadenylation-Mediated Regulation of Gene Expression in *S. cerevisiae*

T60 Veena Halale Manjunath, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

Hidden Message – Nuclear mRNA storage in seeds

13:30 - 13:45 Closing remarks

L7

Structural insights into the cascade of snRNP remodeling steps leading to the formation of a catalytically activated spliceosome

Reinhard Lührmann

Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

The spliceosome, which catalyzes the removal of introns from pre-mRNA molecules, forms anew on each pre-mRNA intron, through a pathway involving multiple, successive assembly intermediates. Early spliceosome formation involves the binding of the U1 and U2 snRNPs to the 5' and 3' ends of an intron, respectively, yielding the A complex. Recruitment of the U4/U5.U6 tri-snRNP leads to the formation of the pre-B complex, which is remodeled into the B complex. The pre-B to B transition, and the transformation of the pre-catalytic B complex into an activated (Bact) spliceosome, involves extensive protein exchanges and RNA rearrangements that lead to the formation of a catalytically active U2/U6 structure. Our cryo-EM structures of pre-B, B and several distinct pre-Bact assembly intermediates reveal an intricate cascade of highly coordinated structural changes during the activation phase of the human spliceosome. They also reveal unprecedented, large-scale translocations of proteins and entire RNP domains, with RNA helicases and kinases acting as driving forces. In addition, our studies reveal the molecular mechanism whereby formation of a catalytically active U2/U6 RNA network is facilitated by spliceosomal proteins, with a conformational change in the scaffold protein PRP8 playing a key role in facilitating its final 3D folding.

10:15 - 11:30 Morning Session I

Transcription and mRNA Processing

T49

Structural basis of the Integrator complex assembly and association with transcription factors

Razew M.[1]; Fraudeau A.[1]; Pfleiderer M.M.[2]; Linares R.[1]; Galej W.P.[1]

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The Integrator complex is a multisubunit protein complex responsible for the premature transcription termination of coding and noncoding RNAs through its RNA endonuclease and protein phosphatase activities. In recent years, significant progress has been made in elucidating its architecture, including structures of the Integrator complex bound to paused elongating RNA polymerase II (RNAPII). Emerging structural and biochemical insights suggest that Integrator is organized into distinct functional modules that cooperate to regulate RNAPII at promoter-proximal sites. However, how Integrator assembly and recruitment are regulated, as well as the functions of many of its core subunits, remain unclear. To address these questions, we recently reported cryoEM structures of two Integrator subcomplexes, along with an integrative model of the fully assembled Integrator bound to the RNAPII-paused elongation complex (PEC). This structural model supports emerging evidence that specific transcription factors interact with Integrator subunits to fine-tune its recruitment and activity. We leveraged recent advances in machine learning, particularly the AlphaFold2 protein structure prediction algorithm, to perform an in silico protein-protein interaction screen between Integrator subunits and over 1,500 human transcription factors (TFs). This analysis identified two TF-binding sites within the Arm/Tail module of Integrator, and we experimentally validated some of these uncovered interactions. This mode of interaction may be more general and could extend to other chromatin readers and remodelers, further increasing the complexity of Integrator's interaction networks. I will summarize our recent progress in structural and functional studies of the Integrator complex and present new insights into its association with auxiliary factors.

T50

SETD2 methyltransferase activity aids gene definition by promoting correct transcription initiation and termination

Magda Kopczyńska[2,3]; Chihiro Nakayama[1]; Agata Stępień[2,3]; Takayuki Nojima[1]; Kinga Kamieniarz-Gdula[2,3]

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SETD2 is a key histone methyltransferase responsible for depositing histone H3 lysine 36 trimethylation (H3K36me3). Loss of its enzymatic activity has been implicated in various cancers, including renal cell carcinoma (RCC). In RCC, SETD2 mutations have been linked to defects in transcription termination, though the underlying mechanism remains unclear. Here, using nascent transcriptomics in RCC patient-derived cells and SETD2 knockouts, we show that SETD2 loss leads to delayed transcription termination, but only in a subset of genes. POINT-seq analysis reveals widespread transcriptional readthrough beyond canonical termination sites upon SETD2 depletion, affecting 15–24% of genes. This readthrough may result in transcriptional interference, disrupting the expression of downstream genes. Mechanistically, we demonstrate that defective termination following SETD2 loss is driven by a combination of increased cryptic transcription initiation and impaired 3' pre-mRNA cleavage. In contrast, genes that maintain proper termination show significant downregulation. Additionally, we find that SETD2 leads to transcriptional readthrough independently of alternative polyadenylation, underscoring the distinction between transcription termination and 3' end processing. Our findings highlight SETD2 as a crucial regulator of transcriptional integrity, preventing cryptic initiation and promoting efficient 3' end processing.

T51

Comprehensive mapping of active transcription by human RNA polymerase III reveals a rate-limiting role for transcription termination

Jan Mikołajczyk[1]; Ameen Kollaran[1]; Jonas Weidenhausen[2]; Christoph W. Muller[2]; Tomasz W. Turowski[1]

[1] Institute of Biochemistry and Biophysics PAS, Warszawa, Poland; [2] European Molecular Biology Laboratory, Heidelberg, Germany

Eukaryotic organisms rely on RNA polymerase III (RNAPIII) to produce 5S rRNA, tRNAs, U6 snRNA, and other small untranslated RNAs. Unlike RNAPII, which transcribes long genes, RNAPIII transcribes relatively short units of 75-300 nucleotides in length and utilizes intragenic promoters as well as a unique termination mechanism. Most highly expressed RNAPIII genes such as tRNAs rarely provide enough room to accommodate more than two elongating polymerases at a given time. At the same time these genes need to accommodate the high RNAP flux required to keep up with cellular demand. These specific constraints suggest kinetics that might differ significantly from those typically observed in RNAPII-transcribed genes. In this study, we utilize the first complete genome-wide mapping of actively transcribing RNAPIII in the K562 human cell line using UV-crosslinking and stringent purification to reveal the kinetics of tRNA transcription in human cells. Our data show that only 50% of tRNA genes are functional, with significant variability in expression, frequent transcriptional readthrough, and unique dynamics across units. We examined genomic and chromatin attributes linked to tRNA gene activity. Despite the short gene length, transcription elongation rate on tRNA genes is variable and dependent on nascent transcript folding. Overall elongation rate tends to drop with distance, eventually culminating in major terminator-associated pausing. This led us to hypothesise that termination is the rate-limiting step in tRNA transcription, which we validated using reporter constructs. Thus, termination efficiency significantly influences tRNA expression by directly regulating gene output. In this light terminator readthrough can be considered a rescue mechanism, allowing RNAPs that failed to release the transcript and clear the terminator to run off.

T52

The by-product of spliceosomal first-step catalysis protonates adjacent catalytic triplex residues, promoting transition to the second step

Bogdan Cichocki; Ishani Sengputa; Katarzyna Eysmont; Magda Konarska

IMol, Polish Academy of Sciences, Warsaw, Poland

The spliceosome catalytic triplex is crucial for catalysis, coordinating catalytic magnesium ions and juxtaposing the two intron substrate sites bound by U2 and U6 snRNA segments for the first step catalysis. In the hydrolytic group II introns the product of the first step, a single H^+ ion, protonates the central cytosine nucleobase of the third triple of the catalytic triplex, destabilizing it and facilitating progression to the second step of splicing (Manigrasso J. et al., 2020). Using *S. cerevisiae* yeast genetics, we describe an analogous mechanism operating during the first step branching catalysis by the spliceosome. We tested all possible nucleobase combinations of the U2-U23:U6-A59•A53 triple by monitoring yeast growth. Mutations of the central U6-A59 nucleobase to non-protonable G or U residues (A59g/u) yield inviable or dominant-negative phenotypes, consistent with the group II introns results. We hypothesize that the inviability of A59g/u alleles results from the formation of hyperstable U23:g/u59•A53 triples, blocking the exit from the first step conformation. Indeed, *prp16-302* or *prp8* first step alleles (that stabilize the first step conformation) exacerbate growth phenotypes of most nucleobase combinations of this triple, whereas U6 alleles that suppress *prp16-302* defects rescue their inviability. Importantly, second step prp8 alleles that stabilize the second step spliceosome conformation, rescue defects of several triple combinations. Strikingly, another nucleobase of the same triple, U6-A53, may also become protonated during the first step catalysis. The A59g/u inviable combinations can be rescued by more readily protonated A53c alleles, and conversely, inviable combinations containing A53g/u mutations can be rescued by A59c. Together, our results strongly suggest that protonation of U6 pos. A53 or A59 ensures the proper balance of stabilities between the first and second-step conformations of the catalytic center. We propose that, by analogy to group II introns, protonatable nucleobases in the U23:A59•A53 triple act as sensors, determining the precise timing of the first step catalysis and facilitating progression to the second step. This strikingly elegant mechanism allows the spliceosome – the enzyme that catalyzes two reactions within one catalytic center – to synchronize the catalytic event with the necessary rearrangement of the catalytic core.

T53

Implications of spliceosome control during the malignant hematopoietic activation

Ankita Kumari, Maciej Ciesla

IMol Polish Academy of Sciences, Warsaw, Poland

Aging expands hematopoietic stem cells, increasing their susceptibility to somatic mutations that drive clonal hematopoiesis of indeterminate potential (CHIP). By age of 70, CHIP affects nearly 10% of the population. Though clinically silent, it increases the risk of hematologic malignancies, including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). CHIP also associates with higher rates of mortality from cardiovascular disease, atherosclerosis, myocardial infarction, and diabetes. At the molecular level, CHIP-associated mutations disrupt epigenetic regulation, genomic integrity, kinase signaling, and pre-mRNA splicing. Splicing rapidly alters the cellular proteome by selecting different protein-coding exons. Our recent work shows that MDS patients upregulate the splicing factor SF3B1, which preserves correct splicing of DNA damage repair genes and acts as an anti-leukemic barrier. To model CHIP, we knock down DNMT3A using RNA interference (siRNA) in hematopoietic stem and progenitor cells (HSPCs) and generate a CRISPR-based DNMT3A knockout for transplantation studies. By comparing HSPCs from young (3-month-old) and aged (>15-month-old) mice, we assess how age of donor cells affects CHIP progression. We profile DNMT3A-KD-driven immunophenotypes and link them to changes in candidate splicing factors (cSFs). We observe that induction of CHIP in young HSPCs lowers U2 snRNP splicing factor mRNA. In contrast, aged HSPCs show no change. We demonstrate ex vivo CHIP modeling and show that cSF deregulation depends on age. Identifying cSFs may help develop strategies to reverse or mitigate CHIP-related phenotypes.

T54

Immediate early splicing after T cell activation is controlled by temporal phosphorylation of hnRNPC2

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Upon activation, T cells undergo rapid changes in gene expression, leading to their functional modifications. Some of these genes are expressed transiently, rapidly, and independently of de novo protein synthesis, classifying them as immediate early genes (IEGs). Our study aims to elucidate similar mechanisms regulating splicing machinery that we term immediate early splicing (IES). Using RNA-seq of nascent transcripts, we identified transient intron retention (IR) events shortly after T-cell activation. These IR events arise within minutes post-stimulation, return to baseline levels within hours, and occur independently of de novo protein synthesis. IES was observed among genes encoding translation factors, including eukaryotic initiation factor eIF5A. This mechanism requires phosphorylation of heterogeneous nuclear ribonucleoprotein C (hnRNPC), which follows a transient kinetic profile and, like many IEGs, is governed by the MEK-ERK signaling pathway. hnRNPC exists in two isoforms, hnRNPC1 and hnRNPC2, with the phosphorylation site present exclusively in hnRNPC2. By increasing the hnRNPC2 levels, we observed enhanced and accelerated IR in eIF5A during early T-cell activation. IES is T-cell specific, as it was not detected in other tested cell lines. Inhibitor-based assays suggest that T cell-specificity is mediated by PKC-θ, a kinase uniquely expressed in these cells. Furthermore, we observed an hnRNPC2-dependent reduction in de novo translation in the hours following T-cell activation. This reduction is mediated by the IES switch, as inducing the IES-specific isoform of eIF5A alone suppresses global translation. Our findings establish a paradigm for rapid and transient alternative splicing during immune activation. A deeper understanding of IES in T cells could lead to novel therapeutic targets, particularly in cancer immunotherapy and autoimmune disease treatment, and facilitate the development of RNA-based therapies capable of modulating immune responses at the post-transcriptional level.

12:00 – 13:15 - Afternoon Session II

Transcription and mRNA Processing

T55

Antagonistic effects of uridylation and deadenylation shape poly(A) tail profiles in Arabidopsis

Quentin Simonnot; Pietro Giraudo; Léa Gerber; Elodie Ubrig; Jackson Peter; Heike Lange; Hélène Zuber; Dominique Gagliardi

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Uridylation promotes the degradation of mRNAs that have undergone deadenylation. We previously identified URT1 as the main enzyme uridylating mRNAs in *Arabidopsis thaliana*. URT1 promotes the 5' to 3' degradation of mRNAs, and prevents the accumulation of excessively deadenylated mRNAs to avoid the biogenesis of illegitimate siRNAs that can silence endogenous mRNAs and therefore strongly impact growth and development. To further explore the relationship between uridylation and deadenylation, we used Oxford Nanopore Technologies (ONT) sequencing to compare mRNA polyadenylation profiles and uridylation status between wild-type plants and the *urt1* and *ccr4a/b* mutants, respectively affected in mRNA uridylation and deadenylation. In wild-type plants, both global and intergenic poly(A) tail distributions show regularly-spaced peaks corresponding to PABP footprints. The shortest and most prominent peak is centered around 20 As, a size smaller than observed for *C. elegans* and human mRNAs. Interestingly, the accumulation of the 20 A peak is enhanced in *urt1* while severely decreased in *ccr4a/b* mutants. In addition, overexpression of URT1 induces a shift towards longer poly(A) tails and therefore mimics the loss of CCR4A/B. Finally, combining the *urt1* and *ccr4a/b* mutations restores the wild-type distribution of poly(A) tails. We therefore propose that antagonistic effects of uridylation and deadenylation participate in shaping the poly(A) tail profiles of Arabidopsis mRNAs, revealing a new role of uridylation. Of note, the shift in mRNA poly(A) distribution between *urt1* and *ccr4a/b* is much more prominent in rosette leaves than in flower buds, indicating that the equilibrium between uridylation and deadenylation is modulated during development. This research was funded by grants ANR-10-IDEX-0002, ANR 20-SFRI-0012 and ANR-17-EURE-0023 from the Agence Nationale de la Recherche (ANR), France.

T56

CFIm-Complex-Driven Alternative Polyadenylation Controls mRNA Length, Stability, Localization and Protein output in colon and other cancers

Maciej Jerzy Smialek, Aleksei Mironov, Selene Stacchi, Lea Mues, Niels Schlusser, Meric Ataman, Nitish Mittal and Mihaela Zavolan

Biozentrum, University of Basel, Basel, Switzerland

Alternative polyadenylation (APA) generates transcript isoforms with distinct 3' untranslated regions (3'UTRs), impacting mRNA stability, localization, and translation. The Cleavage Factor Im complex—CPSF5 (CFIm25), CPSF6 (CFIm68), and CPSF7 (CFIm59)—plays a central role in APA site selection. However, its role in spatial gene regulation and cancer remains poorly defined. We performed siTOOLs-mediated knockdown of CPSF5, CPSF6, and CPSF7 in HCT116 (colon cancer) and HepG2 (liver cancer) cell lines. RNA-seq analysis revealed widespread 3'UTR shortening in CPSF5/6-depleted cells (2184 and 2095 genes, respectively), with smaller effect from CPSF7. APA-induced isoform shortening was largely uncoupled from changes in transcript abundance, supporting recent findings that mRNA length and abundance are orthogonal regulatory axes. Gene ontology analysis of APA-affected transcripts identified enrichment in cancer-relevant pathways, including p53 signaling, nucleocytoplasmic transport, and ribosome biogenesis. Among the transcripts with shortened 3'UTRs, CD47 exhibited enhanced mRNA stability and striking mislocalization of its encoded protein—from the plasma membrane to the endoplasmic reticulum—following CPSF5/6 depletion, as well as after CD47 long 3’UTR isoform depletion. Confocal microscopy confirmed this redistribution, implicating APA in controlling spatial protein targeting. Additionally, ERK1/2 signaling was altered, with p-ERK condensates dissolving upon APA disruption. Other APA targets include NET1, a long-3'UTR isoform previously linked to apical-basal localization in colon epithelia. To expand these findings, we are developing a method to isolate organelle-specific RNAs using antibody-based FACS sorting of cellular compartments (e.g., nucleus, ER, mitochondria, lysosomes, proteasome, TIS granules, plasma membrane, and cytosol), followed by isoform-resolved RNA-seq. This approach will enable subcellular mapping of 3'UTR isoform localization under native and CPSF-depleted (shortened 3’UTRs) conditions. Our data reveal a conserved function for CPSF5/6 in regulating APA and mRNA localization across cancer cell types, suggesting that APA serves as a spatial code for correct protein targeting. These findings underscore APA’s therapeutic potential in modulating oncogenic protein distribution and cellular architecture in colon and other cancers.

T57

Tracking poly(A) tail diversity with Ninetails: mixed tails from cells to mRNA therapies

Natalia Gumińska[1], Katarzyna Matylla-Kulińska[1,2], Agnieszka Czarnocka-Cieciura[1]; Paweł S. Krawczyk[1]; Wiktoria Orzeł[1,2]; Zuzanna Mackiewicz[1]; Aleksandra Brouze[1]; Seweryn Mroczek[1,2]; Andrzej Dziembowski[1,2]

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Poly(A) tails are essential for mRNA stability and translation. Once believed to consist solely of adenosines, poly(A) tails are now known to also contain non-adenosine nucleotides. The advent of Oxford Nanopore Direct RNA Sequencing provided a framework to measure poly(A) tail lengths without amplification bias. However, until recently, it was impossible to gain insights into the nucleotide composition of poly(A) tails. To address this gap, we developed Ninetails, a deep-learning-based approach that enables precise detection and quantification of non-adenosine residues in poly(A) tails at the single-molecule level. Using this approach, we performed a comprehensive, cross-species and tissue-wide exploration of non-adenosine distributions in poly(A) tails. Our study covers multiple organisms, including yeast, mammals, algae, and plants, as well as various murine cell types and tissues such as the brain, liver, macrophages, B and T cells, pancreas, and germ cells. This revealed distinct, functionally relevant patterns – ranging from terminal non-adenosine enrichment in spermatogenesis-related transcripts to semi-templated poly(A) tails in key regulatory mRNAs and increased non-adenosine incorporation in transcripts processed by TENT-family nucleotidyltransferases. These findings suggest that non-adenosine modifications contribute to an additional, yet underappreciated, layer of post-transcriptional regulation. In addition to endogenous mRNAs, we investigated synthetic RNA molecules, including mRNA therapeutics. We found that poly(A) tails in these constructs undergo compositional changes over time in cells. Furthermore, we demonstrate that different mRNA manufacturing processes influence poly(A) tail purity, highlighting the need for enhanced quality control standards in RNA therapeutics. By uncovering widespread poly(A) tail heterogeneity across species and applications, our work provides new insights into the role of non-adenosines in RNA biology and offers a foundation for optimizing the stability and performance of therapeutic mRNAs.

T58

The role of PCF11-similar proteins in alternative polyadenylation and flower development in Arabidopsis thaliana

Mateusz Bajczyk[1]; Lukasz Szewc[1]; Monika Jozwiak[1]; Dawid Bielewicz[2]; Łukasz Grewling[3]; Zofia Szweykowska-Kulinska[1]; Artur Jarmolowski[1]

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Eukaryotic messenger RNA (mRNA) is produced from the primary transcript (pre-mRNA) through extensive processing steps, including splicing, 5' cap, and 3' poly(A) tail addition. The poly(A) tail protects the mRNA from degradation and is required for translation initiation. About 70% of Arabidopsis genes have more than one polyadenylation site and alternative polyadenylation (APA) can alter the length and composition/information encoded in the mature transcript. In plants, one of the best-studied processes affected by APA is the control of flowering time. In Arabidopsis thaliana, PCF11-similar protein 4 (PCFS4), a homolog of yeast polyadenylation factor protein 11 (PCF11), is an important factor involved in this process. Knockout pcfs4 mutants show a delay in flowering time. However, there are three other PCF11-like proteins in A. thaliana: PCFS1, PCFS2, and PCFS5, which have not been characterized. We have shown that the pcfs2 mutant has an opposite effect to the pcfs4 mutant – it accelerates flowering time. Using PCFS coimmunoprecipitation followed by LC-MS/MS we identified interactome of all PCFS proteins. These data revealed that PCFS2 and PCFS4 are associated with polyadenylation machinery, RNA polymerase II, chromatin remodeling factors and factors involved in m6A deposition. In addition, our data showed that plant PCFS proteins can form homo and heterodimers. Interestingly, some of the double mutants (pcfs1 x pcfs4; pcfs2 x pcfs4) showed more severe phenotypes, including aberrant development of the male part of the flower, leading to strong herkogamy and pollen abnormalities. This phenotype is similar to a mutant of another polyadenylation factor, CstF64. Using FRET-FLIM, we showed that all A. thaliana PCF11-like proteins interact with CstF64. Using PAT-seq (Poly(A)-Tag Deep Sequencing) we showed that the distal polyadenylation site is mostly selected in both pcfs and cstf64 mutants compared to wild-type plants. We also found that the male part of the flower is significantly affected in plants with only slightly altered levels of CstF64. In the analyzed mutants, genes encoding pollen envelope proteins have altered polyadenylation. This strongly suggests that PCF11-similar proteins and CstF64 are important factors for proper polyA site selection, which is particularly important for the development of the male part of the flower.

T59

Decoding mRNA Fate: Poly(A) Tail Dynamics and the Role of Puf Proteins in Deadenylation-Mediated Regulation of Gene Expression in S. cerevisiae

Agnieszka Czarnocka-Cieciura [3], Agnieszka Tudek [2], Jarosław Poznański [2], Matti Turtola [5], Rafał Tomecki [2], Paweł S Krawczyk [3], Seweryn Mroczek [1;3], Wiktoria Orzeł [3], Upasana Saha [4], Torben Heick Jensen [4], Andrzej Dziembowski [1,3]

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Cytoplasmic poly(A) tail deadenylation is a key determinant of mRNA stability, influencing transcript degradation and gene regulation. To investigate this process, we performed Direct RNA Sequencing (DRS) using Oxford Nanopore Technologies of *S. cerevisiae* mRNAs under steady-state and stress conditions (Czarnocka-Cieciura and Poznanski et al. EMBO J 2024). Our analysis led to the development of a numerical model estimating the cytoplasmic deadenylation rate at 10 A/min. A complementary approach, based on poly(A) tail quantiles, confirmed a strong correlation between deadenylation and mRNA decay rates. A key discovery was the unexpected stability of ribosomal protein-coding mRNAs (RPG mRNAs), despite their short poly(A) tails. Our findings reveal that RPG mRNA degradation is not solely dependent on deadenylation but also requires active nuclear export and translational control, challenging the classical view that poly(A) tail length universally determines mRNA half-life. Our work also demonstrated that deadenylation rates vary across functional transcript groups and were influenced by codon optimality but also many other factors, whose contribution is far less understood at the transcriptome-wide scale. Thus, as a next step in our research, we sought to examine mechanisms that modulate deadenylation kinetics more closely. We decided to focus on a special group of RNA-binding proteins that interact with the Ccr4-NOT complex. In S. cerevisiae yeast, there are six proteins belonging to the Pumilio-homology domain family. They possess an evolutionarily conserved Pumilio homology domain, which enables them to recognize specific motifs in mRNA sequences. According to the generally accepted model, Puf proteins bind the mRNA molecule and subsequently recruit the Ccr4-NOT complex which shortens its poly(A) tail. Using DRS, we obtained poly(A) tail distribution profiles in double and single deletion mutants of Puf proteins. This allowed us to shed new light on their substrate specificity, interactions with Ccr4-NOT, and functions in eukaryotic cells.

T60

Hidden Message – Nuclear mRNA storage in seeds

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The process of seed maturation is essential for preparing seeds for desiccation and subsequent germination. During this phase, seeds accumulate not only storage materials but also mRNAs that are translated immediately after water uptake. Germination studies involving Pol II transcription inhibitors indicate that seeds can depend on these stored mRNAs for a considerable time after water uptake. However, the mechanisms and place of mRNA storage in dry seeds remain hidden, with recent research suggesting their association with ribosomes. We detected mRNA accumulation in the cell nucleus of dry Arabidopsis thaliana seeds. This suggests a new mechanism for mRNA storage in seeds. Intriguingly, the nuclear mRNA accumulation is significantly stronger in the pab double mutant, which lacks two of three Arabidopsis poly(A)-binding proteins. Phenotypic analysis of pab mutant seeds revealed low dormancy and high germination kinetics. In agreement, transcriptomic analysis of dry seeds showed altered gene expression with the upregulation of mRNAs that are usually observed early during germination. We hypothesise that the physiological changes in pab mutant seeds germination are linked to nuclear mRNA accumulation. I will address the long-standing question about where mRNA is stored in seeds.

Posters

P1

Relationship between miR-125b axis and stage of liver fibrosis in male patients with primary sclerosing cholangitis.

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Introduction: Chronic cholestatic liver diseases, such as primary sclerosing cholangitis (PSC), involve immune-mediated damage to the biliary tree leading to hepatic insufficiency and fibrosis. miR-125b is a modulator of immune responses and has been associated with liver inflammation and tumorigenesis. miR-125b directly targets the transforming growth factor beta (TGF-β), a central mediator of fibrogenesis, while apelin (a negative regulator of the TGF-β pathway) exerts antifibrotic effects. miR-125b regulates the androgen receptor (AR). AR signaling plays important roles in normal liver function and in progression of liver diseases.

Aim: The purpose of this study was to examine the expression of miR-125b, TGF-β, AR, and apelin in different stage of liver fibrosis among male with PSC.

Methods: We analysed miR-125b and TGF-β mRNA expression in serum and peripheral blood mononuclear cells (PBMCs) from male PSC patients (n=43) and healthy controls (n=10) using RT-qPCR. Serum TGF-β, AR, and apelin concentrations was measured using ELISA kit (PSC: n=36, and healthy control: n=10).

Results: The serum miR-125b level was higher in patients with advanced fibrosis than early fibrosis ($p=0.04$) and the expression of miR-125b positively correlated with FibroScan results (r=0.5, $p=0.006$). In PBMC, the expression of TGF-β mRNA was significantly enhanced ($p=0.02$). The level of androgen receptor was lower in men with PSC than in age-matched healthy controls ($p=0.02$), and AR positively correlated with miR-125b expression (r=0.5, $p=0.08$).When the stage of fibrosis was taken into consideration we noticed that the levels of miR-125b, AR and TGF-β were higher in serum from male patients with advanced fibrosis than with early fibrosis ($p=0.04$, $p=0.04$, and $p=0.007$, *respectively*). In contrast, the level of apelin was lower in serum from male patients with advanced fibrosis than early fibrosis ($p=0.04$). The negative correlation was observed between the level of apelin and FibroScan results (r=-0.5, $p=0.05$).

Conclusion: In males patients with primary sclerosing cholangitis, the overexpression of miR-125b was associates with level of androgen receptor, and also TGF-β, apelin and stage liver fibrosis. We demonstrated that miR-125b can be an upstream regulator of androgen receptor, which may be related to liver fibrosis in male patients with PSC.

P2

Direct tRNA sequencing as a new tool to analyse modifications in tRNA at the uridine 34 position.

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The measurement of tRNA modifications with single transcript resolution has only been possible for a few modifications due to the lack of available methods [1]. This limitation does not allow for the advancement of basic research studies on the dynamic nature of tRNA modification, nor for the development of advanced diagnostic tools for several known tRNA-dependent human diseases.

Oxford Nanopore Technology (ONT) has great potential for developing strategies to accurately identify the modification status of the tRNA anticodon loop (ACL), which encompasses the most complex modifications on uridine-containing RNA motifs.

We have investigated the efficacy of nanopore technology to discriminate between complex modifications of uridine 34 in tRNA, which affect the base-calling properties of neighbouring bases and are therefore difficult to accurately predict. The uridine at position 34 is modified to either 5-carbonylmethyluridine (xcm5U34) or 5-carbonylmethyl-2-thiouridine (xcm5S2U34) [2].

We have developed new methods to chemically and enzymatically synthesise single modified tRNA molecules with modifications at the anticodon loop [3]. Nanopore technology captures the features produced by uridine with and without a thiol group when present on synthetic tRNA molecules [3]. We validated our method using LC-MS and biological tRNAs isolated from S. cerevisiae.

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1. Lucas et al. Nat Biotechnol 2024

2. Cappannini et.al. NAR 2024

3. Kusmirek et.al. bioRxiv 2024

P3

Study on liverwort-specific miRNAs steering Marchantia's developmental destiny

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The developmental processes must be meticulously controlled at all stages of a plant's life cycle to ensure proper sexual organ development and reproductive success. This relies on complex genetic networks, the intricacies of which remain largely unknown in the non-vascular plants. To investigate the role of miRNAs in the sexual reproduction of Marchantia polymorpha, we conducted a brief study on the expression patterns of various liverwort-specific miRNAs.

One of these miRNAs, MpmiR11796, exhibited differential expression across Marchantia's vegetative and reproductive organs, with notably high accumulation in the female gametangiophore – archegoniophores. Genomic database analyses along-with 5'-RLM and 3'-RACE experiments revealed that MpMIR11796 gene represents an independent intron-less transcriptional unit (TU), although sharing sequence overlap with a protein-coding gene. A strong MpMIR11796 gene promoter activity was observed in the pegged rhizoids located within digitate rays and stalk of archegoniophores using histochemical GUS staining. Furthermore, CRISPR-Cas9 male Δmpmir11796ko plants produced less rhizoids during gemmae development, while, female Δmpmir11796ko plants showed reduction in thallus and archegonial receptacle size, reduced stalk length, and abnormalities in egg cell development. These observations suggest that MpmiR11796 plays a regulatory role in the overall development of Marchantia and may have a potential impact on fertilization efficiency.

Another miRNA, MpmiR11887 had exceptionally high expression in the male gametangiophore – antheridiophores. Genomic database and experimental analyses revealed that MpMIR11887 gene represents an independent TU. Moreover, the pre-MpmiR11887 is located just downstream of short ORF encoding a putative 65-amino-acid protein of unknown function, which may represent a miPeP. Histochemical GUS staining revealed that the MpMIR11887 promoter is active predominantly in the male generative organs i.e. young antheridia and spermatogenous cells of antheridiophores and weakly in the archegonia of archegoniophores. Furthermore, Δmpmir11887ko plants showed larger thallus area during early stages of gemmae development but ultimately reached a size equivalent to wild-type plants after 2-weeks. Additionally, Δmpmir11887ko plants showed early development of antheridiophores with larger antheridial discs and larger size of mature antheridia.

Finally, transcriptome and degradome data analyses identified various potential targets for MpmiR11796 and MpmiR11887. We aim to unravel the developmental pathways that these miRNAs and their mRNA targets govern.

P4

Profiling Androgen-Responsive Circular RNA Molecules in Triple Negative Breast Cancer Cells: An Experimental and Bioinformatic Approach

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Aims: Androgens appear to play an important role in the biology of breast cancer (BC). The effects can partially be attributed to altering the levels of non-coding circular RNAs (circRNAs), which can "sponge" and block microRNA molecules (miRNAs). Herein, we aimed to determine androgen-regulatory circRNAs in BC cell lines representing different subtypes.

Methods: Five breast cancer cell lines were treated with dihydrotestosterone (DHT) for 48 hrs and the levels of circRNAs, miRNAs, and mRNAs were assessed using high-throughput microarrays followed by analyses using various bioinformatics web tools

Results: Cell type- and cell line-specific changes in the levels of the three RNA classes were revealed. Abundant androgen-regulated circRNAs among 4 cell lines originated from FKBP5, ACSL5, and SEC14L genes. These were predicted to target shared miRNAs that can also block shared mRNAs. Several of these gene products were predicted to alter common metabolic pathways, primarily lipid metabolism. However, molecules involved in cell adhesion such as ALCAM, NPNT, EPHA4, and MMP16, appeared to be commonly targeted in three luminal cell lines, supporting the previously reported effect of androgens on BC.

Conclusions: We propose that androgens can regulate the behavior of BCs by altering the levels of non-coding RNAs.

P5

Lamin-Mediated Chromatin Organization and Its Impact on Transcriptional Programs During Epidermal Differentiation

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The mammalian genome consists of ~2 meters long DNA, which needs to be packed into a ~10 micrometer diameter nucleus. This requires a high level of organization, balancing the need for compaction and maintenance of transcriptional activity essential for cell identity, and is achieved through hierarchical chromatin folding into A/B compartments, topologically associated domains (TADs), and lamina-associated domains (LADs), regulated by architectural proteins such as CTCF/cohesin and the nuclear lamina. Lamins A/C and B form a filamentous meshwork beneath the inner nuclear membrane, anchoring heterochromatin, regulating promoter–enhancer communication, and maintaining transcriptional boundaries. Perturbations in Lamin expression disrupt nuclear architecture and interfere with lineage-specific transcription programs.

Epidermal keratinocytes differentiate in a well-ordered sequence from basal progenitors to suprabasal spinous, granular, and cornified layers. This transition is driven by activation of gene clusters (e.g., EDC, KRT I/II), tightly regulated at the transcriptional level through dynamic chromatin looping and spatial repositioning. While RNA-seq studies have begun to unravel transcriptional changes during keratinocyte differentiation, the role of Lamin-mediated genome organization in shaping these RNA expression profiles remains underexplored.

To address this, we have mapped Lamin B and histone modification H3K9me3, H3K27me3, and H3K27ac, as well as chromatin accessibility in basal and suprabasal murine and human keratinocytes. By integrative bioinformatic analysis, we showed how changes in LaminB1 occupancy correlate with transcriptional activity and chromatin accessibility. These data suggest a critical role for LAD repositioning in regulating key epidermal gene programs.

P6

Gas-sensing riboceptors

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In the scientific literature, direct *in vivo* gas-sensing biomolecules that mediate gasocrine signaling are primarily metalloproteins such as the heme-based NO-sensing mammalian gasoreceptor soluble guanylate cyclase and the microbial O_(2)-sensing gasoreceptors FixL kinase and Dos phosphodiesterase.[1,2] However, if the RNA world hypothesis is true, then one of the first gas-sensing biomolecules is potentially RNA (riboceptors or riboswitches), since gases probably existed before the origin of nucleic acids, and RNA probably did not form in a vacuum.[2,3] The identity of gas-sensing riboceptors and riboswitches capable of binding and sensing various essential gasotransmitters (O_(2), N_(2), and etc.,) and nonessential gasotransmitters (HCN, CH_(4), NO, CO, H_(2)S, C_(2)H_(4), NH_(3), and etc.,) or gaseous/volatile signaling molecules are still unknown. The experimental identification of gasoriboceptors can potentially help not only to understand the origin of environmental gas sensing and autocatalytic ribozyme mechanisms in the RNA world hypothesis, but also of the following postulates of gasocrine signaling. 1) All living organisms composed of one or more cells require gasocrine signaling to sense, communicate, survive and propagate. 2) Gasocrine signaling mediated via gasoreceptor proteins (or perhaps riboceptors) is the most essential cellular and inter-organism signaling. 3) All cells and acellular entities arising from or replicating in pre-existing cells require gasocrine signaling.

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P7

Structural and mechanistic insights of Mitochondrial RNA transport

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Mitochondria, ubiquitously present in eukaryotes, have long been known as the cell's powerhouse for their central role in energy production. However, they are now also recognized as key regulators of diverse cellular processes. These processes are tightly regulated and, due to the semi-autonomous nature of mitochondria, often rely on factors encoded in the nucleus. While communication between the nucleus and mitochondria through protein import pathways is well characterized, the mechanisms governing the selective transport of RNA molecules into mitochondria remain poorly understood and very controversial.

We are interested in investigating the structural and functional mechanics of RNA transport into and out of mitochondria. We aim to identify and structurally characterize the protein complexes involved in this process, as well as determine conserved RNA characteristics that are associated with or facilitate this transport.

Recent studies have identified a few protein factors that may be directly or indirectly involved in the mitochondrial transport of RNA. Among these, human polynucleotide phosphorylase (hPNPase) and adenine nucleotide translocase 2 (ANT2) have emerged as key candidates, along with others such as HuR and Bax/Bak. We hypothesize that these proteins may function either individually or as part of a coordinated and regulated network responsible for mitochondrial RNA transport, acting as direct translocons or RNA-binding regulators that facilitate mitochondrial RNA dynamics. Further structural investigation into their mechanisms will be crucial for advancing our understanding of mitochondrial biology in general and mitochondrial RNA metabolism in particular. This study can eventually contribute to the development of novel therapeutic targets for diseases caused by mitochondrial dysfunction or immune dysregulation.

P8

Non-infectious SARS-CoV-2 replicon as a model for testing novel antiviral inhibitors

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RNA viruses are dangerous threats to human and animal health Several of them are significantly hazardous because of their high epidemic or pandemic potential, e.g., influenza, SARS (including SARS-CoV-2), Ebola, Zika, and MERS viruses. Constant changes occurring within the genomes of RNA viruses, including the SARS virus, lead to the emergence of new virus variants and strains. Hence, a better understanding of the SARS-CoV-2 virus biology could help develop more effective drugs against COVID-19. The presented research aims to better understand the role of RNA structure in the replication process of SARS-CoV-2 and to use this knowledge to design potential inhibitory tools. Based on the RNA secondary structure of SARS-CoV-2, we then designed and synthesized a group of modified antisense oligonucleotides targeting the sequences within the N protein coding region, 5'UTR and 3'UTR of SARS-CoV-2 subgenomic RNA and genomic RNA. To test their inhibitory effect, we generated a non-infectious SARS-CoV-2 replicon containing green fluorescent protein (eGFP) that allowed us to validate the oligonucleotide antiviral effect in the HEK293T cell line. Our research demonstrated that the most prominent inhibitory effect was obtained for seven of the nineteen tested oligonucleotides. In addition, four of the best performing ones were tested on a simplified plasmid system and remain their effectiveness on a non-infectious SARS-CoV-2 replicon. Overall, our results suggest that presented here new, modified oligonucleotides designed based on the revealed secondary structure can be potential therapeutic tools against SARS-CoV-2.

P9

Antisense-mediated regulation of the HIF switch in endothelial cells under hypoxia

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Efficient and safe adaptation to prolonged hypoxia in human endothelial cells and many cancers relies on genome-wide transcriptional reprogramming driven by hypoxia-inducible factors (HIFs): hypoxia-inducible factor 1 (HIF-1) and hypoxia-inducible factor 2 (HIF-2). These transcription factors accumulate in response to oxygen deficiency and induce the expression of genes involved in angiogenesis, metabolic reprogramming, and protection against oxidative stress.

Although HIF-1 and HIF-2 share common targets, prolonged hypoxia favors HIF-2–mediated regulation over the HIF-1 signaling that predominates during acute hypoxia. Consequently, HIF-1 accumulates early during hypoxia and is later replaced by HIF-2, a phenomenon referred to as the HIF switch.

Importantly, our previous studies have shown that the HIF switch results from differences in the mRNA stability of hypoxia-inducible factor 1 alpha (*HIF1A*) and endothelial PAS domain-containing protein 1 (*EPAS1*, also known as HIF-2α), with the latter being more stable. Here, I investigate whether natural antisense transcripts complementary to the 3' untranslated region (3'UTR) of *HIF1A* mRNA can destabilize this transcript and thereby contribute to the HIF switch.

In primary human umbilical vein endothelial cells (HUVECs) exposed to hypoxia, I observed that *HIF1A* antisense RNA 2 (*HIF1A-AS2*) – but not *HIF1A* antisense RNA 1 (*HIF1A-AS1*) – can reduce *HIF1A* mRNA levels. Notably, *HIF1A-AS2* is induced by HIF-1, creating a negative feedback loop during acute hypoxia. Furthermore, my data suggests that during prolonged hypoxia, when HIF-1 is absent, the expression of *HIF1A-AS2* is maintained by HIF-2. This ensures that *HIF1A* does not reaccumulate, thereby preventing reversal of the HIF switch.

Finally, my bioinformatic analysis indicates that the expression of these antisense transcripts is tissue-specific and deregulated in cancer cells. Thus, understanding the role of *HIF1A-AS2* in the transition from HIF-1 to HIF-2 may have translational potential in human diseases.

P10

Identification of partners in crime of toxic RNA with expanded CGG repeats

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The expansion of short tandem repeats underlies the pathogenesis of multiple human inherited disorders, including Huntington's disease, muscular dystrophy or amyotrophic lateral sclerosis and frontotemporal dementia. The expansion of CGG repeats in the 5'UTR of fragile X messenger ribonucleoprotein 1 (FMR1) gene within the range of 55-200 triplets causes fragile X-associated tremor ataxia syndrome (FXTAS), and on molecular level evokes toxic RNA gain-of-function and repeat-associated non canonical (RAN) translation. Toxic RNA with expanded CGG repeats (RNAexp) often interplays with multiple proteins at various stages of RNA life, leading to disease progression, therefore particular proteins interacting with RNAexp can be considered as partners in crime and potential therapeutic targets.

Recent technological advances in sequencing and mass spectrometry allowed for development of multiple strategies to investigate the RNA-protein networks. With aim to identify proteins binding to 5'UTR of FMR1 mRNA, we applied two distinctive RNA-centric approaches: in vitro and in cellulo followed by proteomic screens, to understand the mechanism of RAN translation and find new potential targets for therapies for FXTAS.

Using in vitro RNA pull down, we identified 25 proteins significantly enriched on FMR1 RNA containing CGGexp, compared to control RNA, while in cellulo approach based on an RNA tagging system allowed for identification of 32 proteins that bind to RNAexp. Although results from both screens only partially overlapped, the proteins previously described as binding to RNAexp were identified in two datasets. Selected candidate proteins were further stratified using short interfering RNA- based silencing in FXTAS cell models to evaluate their contribution to RAN translation efficiency.

In particular, we found that insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3) binds directly to the 5'UTR of FMR1 RNA, independently of CGG content. Knockdowns of IGF2BP3 decreased the biosynthesis of CGG repeats RAN translation product-FMRpolyG. Secondly, we found that a ribosomal protein RPS26 and its chaperone TSR2, modulate FMRpolyG production and its toxicity.

Summarizing, two RNA-centric approaches allowed us to identify and validate the role of two different proteins- IGF2BP3 and RPS26- that positively regulate the noncanonical translation of CGGexp RNA in two distinctive mechanisms.

P11

Revisiting the base pair maximization approach for RNA secondary structure prediction with SQUARNA

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Non-coding RNAs play a diverse range of roles in various cellular processes, with their spatial structure being pivotal to their function. The RNA's secondary structure is a key determinant of its overall fold. Given the scarcity of experimentally determined RNA 3D structures, understanding the secondary structure is vital for discerning the molecule's function. Currently, there is no universally effective solution for de novo RNA secondary structure prediction. Existing methods are becoming increasingly complex without marked improvements in accuracy, and they often overlook critical elements such as pseudoknots. In this work, we introduce SQUARNA, a new approach to de novo RNA secondary structure prediction that overcomes the limitations of the commonly used tools. Our benchmarks demonstrate that SQUARNA outperforms the state-of-the-art methods in both single-sequence and alignment-based predictions. SQUARNA is available at https://github.com/febos/SQUARNA.

P12

Remodelling of NANOS1 RNP interactome during early human germ cell development is induced by an infertility-associated variant

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The NANOS family comprises RNA/protein-binding proteins that are specifically expressed in germ cells and are essential for maintaining germ cell fate and survival across species, primarily by post-transcriptionally inhibiting somatic differentiation. In humans, a NANOS1 variant carrying a double mutation p.[Pro34Thr; Ser78del] (MUT-NANOS1) is associated with a complete absence of germ cells in infertile patients. The aim of this study was to identify and compare the dynamics of the WT- and MUT-NANOS1 RNP interactomes involved in key pathways of human primordial germ cell (PGC) specification and development. A human embryonic stem cell (hESC) model featuring tdTomato reporter-linked NANOS3, a marker for PGC specification, was employed. PiggyBac doxycycline-inducible transgenic stable cell lines for both WT and MUT-NANOS1 were generated. During the in vitro differentiation of pre-mesendodermal (preME, a competent stage for PGC formation) cells, human primordial germ cell-like cells (hPGCLCs) were specified within embryoid bodies (EBs). Cells positive for PGC specification and those overexpressing NANOS1 were subsequently sorted. Enhanced cross-linking immunoprecipitation (eCLIP) assay and RNA-seq experiments were performed to identify NANOS1-bound RNA targets and assess their differential expression. Additionally, NANOS1-bound proteins were co-immunoprecipitated, identified, and quantified via mass spectrometry. MUT-NANOS1 expression resulted in the upregulation of canonical WNT signalling mRNA targets bound by NANOS1 at the germ cell stage, notably β-catenin, which activates extracellular matrix adhesion targets, thereby promoting the transition of germ cells toward mesodermal lineages. This shift was accompanied by an increase in late mesoderm markers and a decrease in early germ cell markers. Proteomic analysis also revealed that MUT-NANOS1 interacts with a distinct subset of RNA-binding proteins compared to WT-NANOS1. These findings suggest that MUT-NANOS1 acquires a gain-of-function effect, directing cells toward the differentiation of mesodermal derivatives rather than germ cells, and thus promoting a more somatic cell profile. Overall, this study underscores the substantial impact of the p.[Pro24Thr; Ser78del] mutation on early germ cell development, potentially elucidating the observed infertility phenotype. This research was supported by a grant from the National Science Centre, Poland, OPUS 2019/35/B/NZ1/01665 to JJ.

P13

miRarchitect: a machine learning-based tool for the rational and customized design of artificial miRNAs

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Targeted inhibition of gene expression is a powerful approach in both experimental research aimed at understanding gene function and therapeutic applications. RNA interference (RNAi) technology provides a diverse set of tools for post-transcriptional gene silencing, including synthetic small interfering RNAs (siRNAs), vector-based short hairpin RNAs (shRNAs), and artificial miRNAs (amiRNAs). Among these, amiRNAs are the most complex, consisting of a target-specific siRNA insert embedded within a natural primary miRNA (pri-miRNA) scaffold. In cells, amiRNAs undergo two-step processing by the Drosha and Dicer endonucleases, ensuring their expression levels remain close to endogenous miRNAs. This unique property makes amiRNAs promising therapeutic candidates, combining long-term expression, specificity, and safety with high efficacy.

Given the crucial role of sequence and structure in amiRNA processing and activity, efficient computational support is essential for their rational design. To address this need, we present miRarchitect, a web server for the customized design of human amiRNAs. A unique advantage of this tool is its use of machine learning to optimize target sequence selection, siRNA insert design, and pri-miRNA scaffold choice, ensuring that the resulting amiRNAs closely resemble endogenous pri-miRNAs in both sequence and structure. The predictive accuracy of miRarchitect was validated in cell culture using TMPRSS-2 as a target gene. miRarchitect is publicly available at https://rnadrug.ichb.pl/mirarchitect

P14

Single seeds exhibit transcriptional heterogeneity during secondary dormancy induction

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Seeds play a crucial role in the survival of plants due to their ability to sustain extreme environments and ability to pick the right time for germination. Some plant species have adopted a bet-hedging strategy to germinate a variable fraction of seeds in any given condition, which increases their reproductive success. In this study, we focused on Arabidopsis thaliana to explore transcriptional variability among seeds, which leads to binary germination/dormant outcomes upon stress treatment. We developed a single-seed RNA sequencing approach to monitor fluctuations in transcriptional heterogeneity during both the stress-induction and recovery phases. Our analysis revealed a set of genes with specific expression patterns, which we used to position individual seeds along a transcriptional gradient of germination competence. Consistent with this, dormancy-deficient seeds (a mutant of DELAY OF GERMINATION 1 gene) exhibited a trend towards increased germination competence. In conclusion, our examination of hundreds of single-seed transcriptomes during treatment highlighted variability in gene expression that reflects germination levels in a population of seeds. We believe that further optimization of single-seed RNA-seq will enhance our ability to identify key regulators of variability levels.

P15

Global analysis of the p53 mRNA structure in vitro and in vivo

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Our study concerns a structural and functional investigation of important regions of the mRNA molecule encoding human p53 tumor suppressor protein, one of the major factors protecting cells from malignant transformation. This idea emerged from the fact that although the impact of p53 protein on cellular homeostasis and carcinogenesis is vital and well established, the contribution of RNA structures present in the untranslated and coding regions of p53 mRNA to the regulation of these mechanisms is poorly addressed. This limits the understanding of fundamentally important, p53-dependent physiology and disease-related processes and the development of new therapeutic possibilities targeting p53 mRNA. We hypothesize that the secondary and tertiary structures in p53 mRNA play crucial roles in p53 protein expression and cancer development.

We are testing this hypothesis through a comprehensive analysis of the secondary and tertiary RNA structures located in the untranslated regions (5' and 3' UTR) and coding sequence of human p53 mRNA, as well as their functional evaluation. We are investigating the impact of cancer-derived mutations on global mRNA conformation, dynamics, and functional significance in the regulation of p53 protein expression. The combination of interdisciplinary approaches, including high-throughput in vitro and in vivo RNA secondary structure probing, cryogenic electron microscopy (cryo-EM), biophysical methods, and functional assays in in vitro and cellular systems, will provide valuable insights into the structure and function of known and previously uncharacterized secondary and tertiary RNA motifs, and create a fundamental resource for targeting the important regions of p53 mRNA for therapeutic interventions. It will also add a meaningful contribution to the basic knowledge of nucleic acids structure and answer important questions regarding RNA structure-function relationship in human biology and disease.

P16

miR-379-3p counteracts cancer cachexia through regulation of purinergic signalling, interferon response and mitochondrial stress.

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Cancer cachexia is a highly prevalent wasting syndrome in cancer patients. The early stages of cancer cachexia are not well understood, including differences between biological sexes. In a mouse model of early cachexia, males and females showed divergent pathways to atrophy: muscle from males showed strong mitochondrial defects, whereas females were characterized by activation of interferon response. miR-379-3p was downregulated during cachexia and low levels of miR-379-3p were associated with poor survival of lung cancer patients. Restoring miR-379-3p levels in mice slowed down tumor growth and prevented muscle loss through regulating P2RY6. miR-379-3p restored mitochondrial content and function, inhibited interferon response, and regulated Ca2+-related and apoptotic markers. We demonstrate a link between the purinergic receptor P2RY6, and dysregulated Ca2+ homeostasis, mitochondrial dysfunction and inflammation during early stages of cancer cachexia. These processes are coordinately regulated by miR-379-3p, which we propose as a novel therapeutic target for cancer patients.

P17

BTAF1/MOT1 homolog in Arabidopsis controls lncRNA transcription.

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BTAF1/MOT1 is a Swi2/Snf2 family ATPase that removes TBP (TATA-binding protein) from TATA-box promoters. It helps to redistribute TBP to other TATA-box or TATA-less promoters. We found Arabidopsis thaliana homolog of MOT1 showing abnormal development, including sterility, strong growth defect, weak seed dormancy and increased spreading of TuMV virus upon infection. Observed phenotypes are consistent with transcriptomic changes of protein coding genes. Additionally, in mot1 we were able to identify over 400 novel, potentially regulatory, non-coding transcripts that were upregulated compared to wt. Importantly, the previously annotated lncRNAs with known function, like APOLO, FLAIL, MAS and asDOG1 were also upregulated in mot1 mutant. We conclude, MOT1 controls non-coding transcription through a mechanism that is conserved between yeast, human and Arabidopsis.

P18

Identification of oxidized transcripts in the roots of soybean seedlings

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The role of chemical modifications of RNA in plants functioning in optimal and under stress conditions is gaining increasing attention. However, knowledge on oxidative modifications is still limited. These modifications are formed non-enzymatically, through the action of reactive oxygen species, wherein the most frequent one is 8-hydroxyguanosine (8-OHG, 8-oxoG).

The aim of present study was identification of transcripts enriched in 8-OHG in the roots of soybean seedlings, by the means of RNA-seq. The poly(A)RNA was purified from total RNA, immunoprecipitated with specific 8-OHG antibody and sequenced using Illumina platform. Thereafter, the pools of total and 8-OHG enriched transcripts were bioinformatically compared.

The results show that oxidation is a massive process occurring in the majority of poly(A)RNAs. The identification revealed 227 genes encoding highly oxidized transcripts (HOTs), which were associated with negative regulation of signalling, stress response and protein folding. In addition, 152 genes encoded lowly oxidized transcripts (LOTs), engaged in functioning of mitochondria, cellular response to stimuli and homeostasis of gibberellic acid.

Further research directions include the comparison of RNA oxidation in plant grown in control conditions and under environmental stresses.

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P19

The Cwc25 and Prp8 interactions stabilize the first step conformation

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Cwc25 is an essential yeast protein required for the first catalytic step of splicing. The cryo-EM structure of the yeast spliceosome C complex (Galej et al., 2016) shows the Cwc25 N-terminus very close to the branch site (BS) – U2 snRNA duplex. Here, we tested Cwc25 interactions at the catalytic center to determine their contributions to stabilizing the BS-U2 duplex and their possible role in selecting the branch site nucleotide. We confirm that small deletions and insertions within the N-terminal segment cause severe defects in splicing, but they do not affect the position of the selected branch site.

Furthermore, we tested whether a long, conserved alpha helix (aa 17-58) helps to position the N-terminus at the BS-U2 duplex, identifying the Cwc25 20-24 aa segment as an interaction site with the Prp8 1585-loop. Deletion of this segment severely affects strain viability and first-step catalysis.

Prp8 contacts all important components of the active site. While most of the protein is quite rigid throughout splicing, the 1585-loop (a.k.a. the α-finger, residues 1575–1598) changes its position and conformation during splicing. In cryo-EM structures that manage to visualize the 1585-loop, it is seen screening the 5' splice site from the branch site nucleophile in Bact, interacting with the U2/intron duplex in C, and stabilizing the active site conformation in C* complex.

Here we identified residues in the 1585-loop that are important for the first step of splicing. These residues interact with the Cwc25 protein, helping to position its N-terminus towards the BS-U2 duplex. Mutating these critical residues destabilizes the 1st step conformation, suppressing the cold-sensitivity phenotype of the prp16-302 allele. Several previously identified second-step Prp8 alleles, *W1575R, S1584F/Y*, are located near the Prp8-Cwc25 interaction site. Thus, the Prp8 1585-loop affects both steps of splicing.

P20

Deciphering novel roles of MBNL splicing factors in RNA metabolism and their relevance to myotonic dystrophy

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The family of Muscleblind-like proteins (MBNL1, 2 and 3) belongs to tissue-specific regulators of RNA metabolism and are known for regulating hundreds of alternative splicing and alternative polyadenylation events by binding to specific sequences within pre-mRNA targets. Moreover, MBNLs are also implicated in the regulation of cytoplasmic localization of particular mRNAs, miRNA processing, mRNAs stability and translatability. Over the years, impaired MBNLs activity has been identified as a key factor in the pathogenesis of myotonic dystrophies type 1(DM1) and type 2(DM2). In these disorders harmful RNAs arise with expanded, RNA hairpin forming CUGexp or CCUGexp repeats. These expanded RNA repeats effectively capture MBNLs to form phase-separated ribonuclear condensates. Sequestration of MBNLs diminishes their functional cellular levels causing a well-studied misregulated alternative splicing of several transcripts in DM1 muscles and neurones leading to embryonic pattern of splicing in postnatal development in DM tissues. In current study we aim to use cutting-edge, global transcriptomic techniques to identify new functions of MBNLs to better understand the abnormalities of gene expression in studied disorders, especially the ones related to their cytoplasmic functions. In order to decipher how MBNLs binding to coding sequences or 3'UTRs affect stability and/or translatability of targeted mRNAs in the cytoplasm we have utilized CRISPR/Cas9 technology to first knock-out two major MBNL1 and MBNL2 paralogs in human HEK293/HeLa Flp-InT-REx cells. These MBNL1/2_KO cells were then used to generate three monoclonal, isogenic and tetracycline-inducible cell lines expressing either (1) normal mEGFP-tagged MBNL1-43 isoform present solely in the nucleoplasm, (2) normal mEGFP-tagged MBNL1-41 isoform localized in the nucleoplasm and cytoplasm or (3) mutant mEGFP-tagged MBNL1-41 isoform engineered with two distinct short nuclear export signals to promote efficient cytoplasmic localization. Using polysome fractionation in sucrose gradient combined with RNA sequencing of monosome, light, and heavy polysome fractions, we identified cytoplasmic MBNL1-dependent target RNAs. Moreover, by comparing alternative splicing profiles between cells expressing MBNL1-41 and MBNL1-43 isoforms, that differ only by the inclusion of alternative exon 5, we observed distinct splicing regulatory activities. These findings may provide new insights into the temporal progression of DM1 pathogenesis and the functional diversification of MBNL isoforms.

P21

Inactivation of Maf1, global negative regulator of tRNA transcription, leads to defects in translation elongation in yeast

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Ribosomes decode mRNAs one codon at a time, and the efficiency of this process is heavily influenced by the availability of aminoacylated tRNAs. In both yeast and mammals, tRNA levels are regulated by the highly conserved protein Maf1. Maf1 controls tRNA synthesis by directly regulating RNA polymerase III transcription, and perturbations to Maf1-mediated regulation are expected to have a downstream impact on mRNA translation. In this study, we used a combination of classical yeast genetics and high-throughput sequencing to determine the effects of Maf1 inactivation on mRNA translation in yeast. In a genetic screen, we identified the translation elongation factor eEF1A as a suppressor of the maf1Δ growth defect. Protein synthesis was decreased in the absence of Maf1, but this phenotype was partially reversed upon eEF1A overexpression. Ribosome profiling revealed increased ribosome density across nearly all mRNAs, suggesting globally slowed translation elongation in maf1Δ cells. Indeed, tRNA abundance was significantly altered in the absence of Maf1, and tRNA species with decreased expression showed increased ribosome pausing over the cognate codon. Finally, Maf1 deletion led to increased translation of genes involved in amino acid biosynthesis. We conclude that Maf1-mediated regulation of tRNA expression is a critical determinant of global translation.

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P22

Short dsRNA, Big Differences: Innate Immune Recognition Across Species

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The poultry industry is the fastest-growing sector in animal agriculture, yet it faces major economic threats due to outbreaks of RNA virus infections, with avian influenza A virus as a prime example. These viruses not only endanger animal health but also pose a zoonotic risk to humans. While innate immunity is well-characterized in humans, translating this knowledge to other species remains a challenge due to evolutionary divergence and distinct ecological contexts.

Our research focuses on innate antiviral responses in chickens and ducks – species central to zoonotic transmission. RNA virus infection typically results in the accumulation of double-stranded RNA (dsRNA), a key pathogen-associated molecular pattern. Recognition of dsRNA by RIG-I-like receptors initiates signaling cascades that drive the interferon response and the expression of interferon-stimulated genes. Additionally, dsRNA sensing activates two main cell growth inhibitory pathways: the PKR pathway, which leads to translation shutdown, and the OAS-RNase L pathway, which degrades both host and viral RNA.

To explore species-specific differences in dsRNA recognition and immune activation, we first conducted both in cellulo and in vitro assays using cells from three species: human, chicken, and duck. Concurrently, we examined the interactome to identify proteins that bind to dsRNA and to detect species-dependent interactions. Interestingly, short, 5'-phosphorylated dsRNAs – potent immune activators in humans – also activate the immune system in duck cells, but fail to trigger a comparable response in chicken cells. These molecules also bypassed the PKR and OAS-RNase L pathways in all studied species. These findings suggest that chicken cells have a reduced ability to detect and respond to certain viral signatures.

These findings raise critical questions: **Are chickens more susceptible to specific RNA viruses due to impaired detection of short dsRNAs? Is their innate immune system inherently limited, or does it depend on alternative, yet unidentified, mechanisms?** By unraveling these interspecies differences in dsRNA sensing, our study advances understanding of antiviral innate immunity in avian species, highlighting potential vulnerabilities relevant to the emergence and prevention of zoonotic diseases.

P23

The SF3A Complex Regulates Cytoskeletal Organization and Myotube Maturation During Myogenic Differentiation

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With this project, we aimed to determine how alterations in splicing downstream to the SF3A complex affects myogenic differentiation and myotube formation.

Alternative splicing (AS) plays a critical role in muscle development by regulating isoform diversity and gene expression during myogenesis. The spliceosome, a dynamic macromolecular complex composed of small nuclear ribonucleoproteins (snRNPs) and associated factors, orchestrates this process. Within the U2 snRNP, the SF3A complex (comprising SF3A1, SF3A2, and SF3A3) is essential for its assembly and catalytic progression. Although alterations in SF3A component expression have been reported in muscle disorders such as Myotonic Dystrophy type 1 and Emery-Dreifuss Muscular Dystrophy, the specific role of SF3A in muscle cell differentiation remains unclear.

To this end, we performed RNAi-mediated knockdown of individual SF3A subunits in C2C12 mouse myoblasts and assessed their capacity to differentiate and form myotubes. We evaluated myogenic markers, cytoskeletal organization, and alternative splicing events by molecular and imaging techniques.

Downregulation of SF3A complex components impaired myotube formation, with SF3A3 knockdown producing the most striking phenotype. Myotubes formed under SF3A3 depletion exhibited abnormal overgrowth, increased branching, and significantly larger diameters compared to controls. These aberrant myotubes displayed reduced expression of differentiation markers such as Myh3 and Myh7, while early myogenic markers (Myf5, MyoD1) remained unaffected. Additionally, SF3A3 knockdown led to pronounced defects in tubulin cytoskeleton organization, suggesting a role in structural integrity.

Our findings reveal that SF3A, and in particular SF3A3, is essential for proper myotube formation and maturation. This function appears to be mediated through cytoskeletal regulation possibly due to the splicing of structural genes. Disruption of SF3A3 compromises myotube architecture and highlights a previously unrecognized link between splicing machinery and muscle cell morphogenesis.

P24

Transcriptional Regulation by Chromatin Looping at Premature Termination Sites

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Premature transcription termination (PTT) has emerged as a critical, yet underexplored, mechanism shaping the mammalian transcriptome. PTT acts as a regulatory checkpoint that influences RNA polymerase II dynamics, modulates RNA abundance, and impacts transcript isoform diversity. A subset of polyadenylation sites, often located within the first intron, can induce transcriptional attenuation, thereby restricting the production of full-length transcripts and altering gene expression outputs. Genomic analyses have revealed that many premature termination sites (PTS) colocalize with binding sites for CTCF and the cohesin complex, key architectural proteins involved in chromatin loop formation and loop extrusion. These observations raise the possibility that chromatin architecture plays an active role in facilitating premature termination and thereby directly regulates RNA biogenesis and transcript fate. The contribution of chromatin loops anchored at PTS to premature termination will be investigated using mouse embryonic stem cells engineered with auxin-inducible degron alleles of proteins essential for loop extrusion and T4ph mNET-seq, a nascent RNA profiling techniques that maps transcription and termination events at single-nucleotide resolution to assess consequences of loop disruption. Integrating nascent transcriptome data with ChIP-seq and chromatin conformation datasets will allow detailed analysis of how spatial genome organization affects RNA Pol II behavior. Validation of this model would imply three-dimensional genome architecture as an integral regulatory layer in transcriptional control. These findings are expected to refine existing models of gene regulation and offer mechanistic insights into disease processes driven by aberrant transcription termination.

P25

MicroRNA Dysregulation in Chondrosarcoma: Identifying Biomarkers for Prognosis and Therapy

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Background:

Chondrosarcoma (ChSA) is a malignant cartilage tumor with limited treatment options and poor survival rates. MicroRNAs (miRNAs) play crucial roles in gene regulation and tumor progression, yet their involvement in ChSA remains insufficiently characterized. This study aims to define the miRNA landscape of ChSA and identify potential biomarkers for disease progression and therapeutic targeting.

Methods:

We performed miRNA profiling using FFPE-derived tumor and adjacent normal tissue pairs from 40+ ChSA patients. Differential expression analysis was conducted to identify significantly dysregulated miRNAs and their downstream gene targets.

Results:

Our preliminary findings reveal strong miRNA dysregulation in ChSA, particularly within the miR-7a-345 and miR-7a-100-122 regulatory networks. Notably, miR-4488 was significantly upregulated (>5 $\log_2$FC, $p < 0.05$). Over 50 miRNAs exhibited a $\log_2$FC >1.5, with the most upregulated being miR-100, miR-134, miR-140, miR-199, miR-214, and miR-98, while the most downregulated included miR-139, miR-142, miR-150, miR-183, miR-223, and miR-744. Functional pathway analysis linked these miRNAs to oncogenic targets, including MYC, NCOA3, BCL2, NHLRC3, NAP1L1, and MTUS1 suggesting their role in ChSA pathogenesis.

Future Directions:

To expand our understanding of miRNA involvement in ChSA, we aim to:

-Increase the sample size to 60 paired tumor-normal samples for a more robust analysis.

-Perform patient-grade stratification to identify miRNAs driving progression from low-grade (G1/G2) to high-grade (G3) ChSA.

-Correlate miRNA expression profiles with clinical parameters such as overall survival (OS), disease-free survival (DFS), and recurrence-free survival (RFS).

-Investigate the potential of identified miRNAs as predictive biomarkers for treatment response and disease progression.

-Validate key miRNAs through functional assays to determine their mechanistic roles in ChSA pathophysiology.

Conclusion:

Our findings highlight the significant role of miRNA dysregulation in ChSA and provide a foundation for future studies to establish clinically relevant miRNA signatures. This research may contribute to developing novel prognostic markers and therapeutic targets for ChSA patients.

Keywords: Chondrosarcoma, microRNA, FFPE, NGS, tumor biomarkers, clinical correlation

P26

Transcriptional regulation of the mitochondrial retrograde pathway in *Candida albicans*

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Retrograde signalling (RTG) is a pathway that responds to dysfunctions of mitochondrial respiration and facilitates the adaptation of the cell to stress. We investigated the RTG pathway in *Candida albicans*, an opportunistic pathogen that, unlike *Saccharomyces cerevisiae*, prefers respiratory metabolism over fermentation, is Crabtree negative, and contains subunits of Complex I (CI) encoded in the mitochondrial genome. *C. albicans* metabolic flexibility, as well as its evolutionary separation from *S. cerevisiae* encompassing several major events (whole genome duplication and loss of CI in Saccharomycetales, change in the universal genetic code and loss of two RTG regulators in Serinales), make it an interesting research model. These differences prompted us to investigate the retrograde response using respiratory-deficient strains of *C. albicans* (lacking CI or ATP synthase), to identify the mechanisms and effectors that differ from those already known in *S. cerevisiae*.

The main regulator involved in the RTG response in yeasts is the heterodimeric basic helix-loop-helix/leucine zipper transcription factor Rtg1-Rtg3. In *S. cerevisiae*, upon mitochondrial dysfunction, the ScRtg1/Rtg3 heterodimer binds to the R-box (5'-GTCAC-3') in the promoters of RTG effector genes, where it activates their transcription. In a search for novel target genes regulated by the CaRtg1/Rtg3 dimer in *C. albicans*, we performed chromatin immunoprecipitation sequencing (ChIP-seq) experiments using CaRtg1-HA in two respiratory-deficient mutants and a wild-type, respiratory-competent strain. We confirmed *in silico* the presence of the canonical CaRtg1/Rtg3 CARbox (5'-GTCA-3') binding motif, and identified pathways enriched for the effectors found in our screen. We verified the most promising candidates by electrophoretic mobility shift assays (EMSA) caused by the CaRtg1/Rtg3 dimer binding in their promoters.

Among targets of the RTG pathway in *C. albicans*, we found genes involved in the glyoxylate cycle, oxidative stress response, alternative respiration and mitophagy. Their activation by the retrograde signalling likely enables the pathogen to survive in unfavourable conditions associated with insufficient respiration.

P27

Role of Polycomb complexes in keratinocyte differentiation

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The epidermis, a stratified epithelial tissue forming the skin's outermost layer, acts as a dynamic barrier essential for protecting the body and maintaining tissue homeostasis. This barrier is maintained through a tightly regulated transcriptional program that governs keratinocyte differentiation from the basal proliferative layer to the suprabasal layers. Key chromatin modifiers, Polycomb Repressive Complexes (PRC1 and PRC2), are known to mediate transcriptional repression through histone modifications, yet their precise roles in epidermal gene regulation remain incompletely understood.

To clarify the contribution of polycomb-mediated transcriptional repression to epidermal differentiation, I used integrative bioinformatics to analyze the transcriptional and epigenetic landscape within the Epidermal Differentiation Complex (EDC), as well as Keratin I and II gene clusters, during mouse development and mature epidermis formation. My findings reveal dynamic transcriptional changes and enhanced deposition of the repressive histone mark H3K27me3 in differentiated keratinocytes, suggesting increased PRC2 activity in terminal differentiation.

Building on these insights, I have established a 3D organotypic culture system using human N/TERT keratinocytes to model epidermal differentiation in vitro to be able to genetically modify the keratinocytes. This platform enables investigation of polycomb-regulated transcriptional networks and the enhancer-promoter architecture underlying keratinocyte maturation.

To functionally investigate the roles of polycomb complexes, I aim to generate CRISPR/Cas9-mediated knockouts of *EZH2*, the catalytic subunit of PRC2, and *RING1A/B*, core components of PRC1 responsible for H2AK119ub deposition. The impact of PRC1/2 depletion on transcriptional regulation, RNA expression profiles, and differentiation potential will be evaluated in both 2D monolayer cultures and 3D epidermal equivalents.

P28

Identification and structural modeling of the novel TTC33-associated core (TANC) complex involved in DNA damage response

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A large portion of the human coding genome remains uncharacterized. Many proteins of unknown function are poorly conserved, and exclusively structural, which hampers, and discourages, functional annotation. Collectively, such proteins add up to 9% of the coding genome, constituting a large gap in our understanding of cell biology. In our work, we characterized TTC33, a 30 kDa protein conserved only in bony vertebrates and composed of tetratricopeptide (TPR) repeats. Comparative label-free mass spectrometry showed that it forms a TTC33-associated (TAN) network of interactions by associating with WDR61, uncharacterized CCDC97, UNG, PP2A-B55α, PHF5A, and the SF3B subcomplex of U2. Through large scale purification and size-exclusion chromatography, we further show that at the heart of TAN is a core complex composed of TTC33, WDR61 and PHF5A. We scrutinize *in silico* structural models of the TTC33 core TPR2-3-4 structure, and describe the features of a TANC complex formation proposed by the AlphaFold 3 algorithm. To expand the structural model we employed molecular dynamics to identify the most stable amino acid contact pairs between complex subunits. Finally, to validate the model we fragmented the TTC33 protein and showed that detachment of TPR4 from TPR1-2-3 impairs recruitment of both WDR61 and PHF5A. We cross-compared the evolutionary conservation of TTC33 protein sequence to the distribution of mutations reported in population studies (gnomAD, 1000genomes, TOPMed), and cancer genomics (COSMIC, TCGA). We showed that somatic mutations found in some cancers co-localize with amino acids crucial for recruitment of WDR61 or PHF5A, and evaluated them experimentally. To identify TTC33's cellular function, we explored the concept of "function by proximity". Although TTC33 forms a complex with WDR61 and PHF5A, both of which are involved in RNA metabolism, our RNA-seq assays revealed only a subtle impact on mRNA levels and splicing patterns. In contrast, depletion or loss of either TTC33 or CCDC97 induced redistribution of p53-S15P, a marker of DNA damage. Accordingly, a comet test showed

increased DNA double-strand breaks in TTC33-depleted cells. We showed that TTC33 protein levels are regulated *in vivo*, and that changes in TTC33 abundance reduced cellular proliferation rate and cell resistance to hydrogen peroxide.

P29

Effective cellular response to dsRNA may occur without the onset of inflammation

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Viral infection of human cells results in the excess of double-stranded RNA (dsRNA) in the cytoplasm. Such dsRNA molecules are either intermediates originating from viral replication or the viral genome itself. The human innate immune response is triggered after dsRNA recognition, which can initiate the cellular defense against the pathogen. Recognition of dsRNAs results in activation of innate immune pathways, which leads to cellular inflammation and inhibition of cell growth. However, we showed that an effective dsRNA response might occur without the onset of inflammation. We proved that pro-inflammatory (RLR-dependent pathway) and cell growth inhibitory mechanisms (OAS/RNase L- and PKR-dependent pathways) could act independently. We found that the 5' ends of dsRNA directed the onset of cellular inflammation. Our results indicated that compared to uncapped transcripts, protection of 5' ends of dsRNA, especially with 2'-O-methylated caps, significantly reduced immunogenicity of the duplex. Finding these non-immunogenic transcripts allowed us to study two main cell growth inhibitory pathways independently of the cell's inflammatory response. We concluded that RNA duplex activated both of them. We found that three of the most common human RNA epitranscriptomic marks – i.e., N6-methyladenosine (m6A), 5-methylcytosine (m5C), and pseudouridine (Ψ) – had only slight influence on the immunogenicity of dsRNA; however, the presence of N6-methyladenosine in the body of RNA caused the duplex relaxation which prevented activation of OAS/RNase L-dependent pathway. All these findings provided novel insight into the precision of innate immunity regulation in human cells upon the appearance of a specific threat.

P30

Investigating the function of N6-methyladenosine RNA modification in barley seedlings during leaf senescence triggered by darkness

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Leaf senescence in crops is a controlled process involving nutrient recycling and cell degradation. Research on the dark-induced leaf senescence (DILS) model in barley seedlings has identified epigenetic factors that regulate this process. Unlike developmental senescence (DLS), which is irreversible, studies have shown that dynamic RNA modifications, particularly the reversible ones, play a significant role in this model. One of the most well-studied reversible RNA modifications is N6-methyladenosine (m6A), which is influenced by a complex interplay of enzymes known as the m6A machinery. These enzymes include RNA methyltransferases, demethylases, and RNA-binding proteins that recognize modified transcripts. Our research focuses on understanding how these reversible m6A modifications regulate induced leaf senescence and their potential for reversibility. In the barley genome, 31 genes related to the m6A machinery were identified, and their expression was analyzed using qRT-PCR. Significant changes in expression were observed for HvMTC, HvALKBH1, HvALKBH9, and HvECT8 during senescence, indicating their involvement in m6A-mediated regulation. Furthermore, the methylated RNA immunoprecipitation sequencing revealed widespread changes in RNA methylation levels in senescing leaves compared to those under normal conditions This research seeks to advance the understanding of epigenetic regulation in stress-induced leaf senescence and enhance cereal crop productivity.

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P31

PIWI Proteins Modulate HIF-1-Driven Endothelial Adaptation to Chronic Hypoxia

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Cellular adaptation to prolonged hypoxia in human endothelial cells is accompanied by the accumulation of PIWI-interacting RNA (piRNA)-like sequences and increased expression of PIWI proteins. In this study, we analyzed next-generation sequencing data from three independent experiments in which primary human umbilical vein endothelial cells (HUVECs) were exposed to varying durations of hypoxia. Our results revealed that the majority of hypoxia-induced piRNA-like RNAs likely originate from degradation of transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs).

Transcriptomic profiling indicated that these piRNA-like RNAs are unlikely to participate in canonical RNA interference (RNAi) pathways. Instead, they appear to play a stabilizing role for PIWI proteins. Biochemical assays demonstrated that elevated levels of PIWI proteins during hypoxia modulate the extent of the hypoxia-inducible factor 1 (HIF-1)-mediated adaptive response.

Knockdown of PIWI in hypoxia-exposed HUVECs led to reduced expression of key HIF-1 target genes, including *SLC2A1* (*GLUT1*), involved in metabolic reprogramming, and *VEGFA* (vascular endothelial growth factor A), a central regulator of angiogenesis. Functional assays further supported the role of PIWI proteins in hypoxic adaptation: HUVECs transfected with small interfering RNAs targeting PIWI proteins exhibited impaired vascular network formation in angiogenesis assays. Interestingly, flow cytometry (FACS) analysis revealed that PIWI knockdown also conferred a pro-survival effect under hypoxic conditions.

Taken together, our findings suggest that PIWI proteins act as modulators of the HIF-1-mediated endothelial response to chronic hypoxia. However, further studies are needed to elucidate the molecular mechanisms underlying PIWI-HIF-1 interactions and their influence on cell fate decisions in hypoxia-exposed endothelial cells.

P32

A specialized TFIIS as a linker between RNA Polymerase II and chromatin in ncRNA transcription

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RNA Polymerase II (Pol II) is responsible for synthesizing all coding (mRNA) and several classes of non-coding (ncRNA) transcripts. However, the mechanisms that regulate the switch between these two transcriptional modes remain incompletely understood. The ciliate *Paramecium tetraurelia* offers a powerful model to study this process, as it features a nuclear dimorphism with distinct transcriptional programs: mRNA is produced in the vegetative macronucleus, while developmentally regulated ncRNAs, as well as long non-coding transcripts, are synthesized from the germline micronucleus and the developing macronucleus respectively, during the sexual cycle. In our previous study, we identified six paralogs of the transcription elongation factor TFIIS in *P. tetraurelia*, each showing distinct expression patterns and nuclear localization. Among them, TFIIS4 was found to be essential for the production of long, developmentally regulated ncRNAs in the early stages of macronuclear differentiation. In contrast, TFIIS1a/c and TFIIS3 are likely involved in mRNA transcription during vegetative growth. These findings highlight the functional divergence among TFIIS paralogs and support their role in modulating Pol II activity in a context-dependent manner. Structural and functional comparisons of TFIIS paralogs provide a promising approach to dissect the regulatory mechanisms governing coding and non-coding RNA transcription. Altogether, this study contributes to a deeper understanding of Pol II plasticity and the dynamic interplay between transcription elongation factors and transcriptional output.

P33

Structure-function studies of the mRNA decapping enzyme of Trypanosoma brucei

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In Trypanosomatids, **mRNA decapping is uniquely performed by ALPH1**, an enzyme from the ApaH family of phosphatases — in stark contrast to all other eukaryotes, which use the Dcp2 Nudix hydrolase. This distinct cap cleavage mechanism sets ALPH1 apart mechanistically from Dcp2 and offers promising opportunities for **trypanocidal drug development and innovative biotechnology applications**.

In this project, we established a robust protein production protocol for *Trypanosoma brucei* ALPH1 and its several truncated versions. This workflow combines bacterial expression with multistep purification to yield high-quality protein. Using SEC-MALS, we found that **ALPH1 forms a homodimer, with oligomerization occurring via its C-terminal domain**. To study its activity, we developed a one-step **enzymatic assay using the direct fluorescent probe m^7GTP-pyrene**, which emits increased fluorescence upon cap cleavage. This assay also enabled us to **demonstrate effective inhibition of ALPH1 by a non-cleavable cap analog**.

We are currently engineering and screening additional variants of the ALPH1 catalytic domain for crystallization trials. **Solving the crystal structure of ALPH1 in complex with cap analogs and inhibitors will provide deeper insights into its reaction mechanism and substrate specificity** — insights that will directly support our ongoing drug development efforts.

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P34

Unveiling the Initial Kinetics of Coronavirus Replication

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Coronaviruses (CoVs) are positive-sense single-stranded RNA (ssRNA+) viruses with 30 kb-long genomes encoding RNA-dependent RNA polymerase (RdRP). Subgenomic RNAs (sgRNAs) are produced via a discontinuous transcription mechanism involving RdRP-mediated template switching. This process is guided by sequence similarity between the 5' UTR and transcription regulatory sequences, and significantly complicates accurate strand-specific quantification. Although, central to viral replication, the early regulation of RdRP-driven transcription remains poorly understood, and the synthesis of negative-sense RNA (ssRNA−) intermediates is poorly understood. In the later stages of infection, this process occurs in double-membrane vesicles (DMVs). However, the necessity of DMV formation for initiating ssRNA(−) synthesis in early infection has not been determined.

In this study, we attempt to address this gap by investigating the synthesis-initiating time of ssRNA(−) and ssRNA(+) alongside the temporal relationship between ssRNA(−) accumulation and DMV-associated protein expression using a dual approach: strand-specific RNA quantification and subcellular localization of replication complexes.

First, we analyzed time-resolved deep RNA-seq data from SARS-CoV-2-infected Vero cells. Positive-sense genomic RNA (gRNA) showed marked accumulation between 8–17 hours post-infection (hpi), whereas negative-sense strands remained consistently low. Building on these findings, we developed a strand-specific protocol to investigate early infection for model CoV, Murine Hepatitis Virus (MHV) in the LR-7 cell line. To enable absolute quantification at the very early time points, the method uses a digital PCR approach (ddPCR). This method allowed for accurate quantification of MHV1 RNA+ and RNA- transcripts with unprecedented time resolution.

To complement RNA measurements, we investigated the localization of viral non-structural proteins NSP3, NSP4, and NSP6—key mediators of DMV formation. Our work will deepen our understanding of how coronaviruses establish replication competence in host cells and identify early steps that may be vulnerable to antiviral intervention.

P35

Targeting HuR-Mediated Regulation of Alpha-Synuclein in Neuronal cell Models of Parkinson's Disease

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Parkinson's disease (PD) is characterized by the pathological accumulation of alpha-synuclein (SNCA), a process associated with neuronal degeneration. Recent studies have implicated the RNA-binding protein HuR (ELAVL1) in the stabilization of alpha-synuclein mRNA, suggesting that targeting HuR-mediated RNA-protein interactions could present a novel therapeutic strategy for PD.

In this study, we investigated the effects of various HuR-targeting small molecules in neuronal models, including SH-SY5Y neuroblastoma cells and rat cortical neurons. SH-SY5Y cells were divided into two distinct subtypes, "S" and "N," and subjected to differentiation protocols. Treatment with different HuR inhibitors was applied to both differentiated and undifferentiated cells, and alpha-synuclein levels were quantified via western blotting and immunostaining. We observed subtype- and differentiation-dependent responses to HuR inhibition. S-type SH-SY5Y cells responded to treatment, even without differentiation, showing a reduction in alpha-synuclein levels. N-type SH-SY5Y cells required differentiation before responding to treatment with reduced alpha-synuclein levels. In rat cortical neurons, treatment with selected HuR inhibitors also resulted in decreased alpha-synuclein levels.

These results demonstrate that the regulation of alpha-synuclein via HuR inhibition is highly dependent on neuronal subtype and maturation status. Our findings underscore the importance of cellular context in the development of RNA-binding protein-targeted therapies for neurodegenerative diseases such as PD and support the strategy of pharmacologically targeting HuR as a promising avenue for future intervention.

Key words: Parkinson's Disease (PD), Alpha-synuclein (SNCA), RNA-binding protein, HuR (ELAVL1), Neuronal models

P36

A tRNA-like sequence in the yeast gene encoding Maf1, a general repressor of tRNA transcription: possible regulatory implications

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Maf1 is a general repressor of RNA polymerase III conserved from yeast to human and plants. The activity of Maf1 is controlled at post-translational level, by phosphorylation of Maf1 protein that specifies its subcellular localization and association with RNA polymerase III complex. Here, we focused on the regulation of Maf1 at post-transcritpional level. The *MAF1* gene of *Saccharomyces cerevisiae* contains intron that is localized at the 5'-terminus of the coding region. We identified a tRNA-like sequence (TLS) that was localized immediately downstream of the 3' intron splice site in the second exon of *MAF1*. Point mutations which destroy a secondary TLS structure but not change coding sequence result in higher levels of *MAF1* mRNA precursor versus mature transcript. A positive effect of TLS on processing of *MAF1* mRNA, indicated by these data, was further confirmed by using the CUP1 splicing reporter system.

Interestingly, TLS has been previously identified in the intron of the *MAF1* gene from *Arabidopsis thaliana* and found to be cleaved by a plant PROPR enzyme, protein only RNaseP, Knowing the ability of PROPR to functionally replace yeast RNP RNase P, we examined whether a TLS in the yeast *MAF1* is a PROPR substrate. An *in vitro* assay performed with the TLS containing fragment of yeast *MAF1* pre-mRNA and recombinant PROPR protein indicated that the cleavage occurs only when the intron sequence is removed. This preliminary result is consistent with the *in vivo* observation that the level of mature *MAF1* mRNA is decreased in the *maf1* intron deletion mutant.

Altogether, our results indicate a multilayer regulatory role of TLS in postrascriptional regulation of Maf1 expression.

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P37

Isoforms of mitochondrial-localized Lsm proteins affect the respiratory capacity of *Saccharomyces cerevisiae* cells

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Mitochondria are essential organelles that perform multiple functions for cell homeostasis. It has been estimated previously that the mitoproteome comprises ~1000 proteins in yeast *S. cerevisiae* and ~1500 in humans. However, more recent studies have identified over 3400 proteins in yeast as mitochondrial or associated with mitochondrial fractions. In addition, it has been reported that several isoforms of nuclear and cytoplasmic proteins that acquire mitochondrial targeting signals (MTS) through N-terminal extensions (NTE), are localized to mitochondria. These proteoforms are generated by the non-canonical translation initiation, usually from non-AUG codons. Although they are synthesized at a much lower level than canonical variants, they can be detected in mitochondria using biochemical and microscopy methods. Preliminary data from previous studies suggest that proteins involved in RNA metabolism, such as subunits of the LSM complexes, are targeted to mitochondria, suggesting that mitochondrial localization may be a more widespread feature among RNA-related factors. Using biochemical and functional assays, we checked mitochondrial localization of specific members of LSM complexes and their impact on respiratory function in yeast. We demonstrated that several Lsm proteins are indeed present in mitochondria. Moreover, depending on the genetic background, strains lacking some subunits of the LSM complexes, are respiratory deficient, suggesting that LSM complexes may have a functional contribution to mitochondrial activity. We speculate that they participate in the processing and/or decay of mitochondrial transcripts.

P38

Mutations in miRNA genes in cancer

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miRNAs are short, noncoding regulatory RNAs that play an important role in many diseases, especially cancer. The essence of cancer is associated with the accumulation of thousands of somatic mutations in its genome. While numerous such mutations have been identified in protein-coding sequences, very little is known about cancer somatic mutations in the noncoding genome, including miRNA genes.

To address this issue, we performed the first comprehensive analysis of mutations in miRNA genes across multiple cancer types. Using large genomic datasets, primarily from over 10,000 cancer samples from the TCGA project, we identified and characterized over 10,000 mutations in miRNA genes, including ones correlated with miRNA levels, cancer severity, and patient survival. We identified the first miRNA genes frequently mutated in cancers, including *MIR142*, which is particularly frequently mutated in hematologic malignancies and is one of the most frequently mutated noncoding elements across all cancers. To characterize the consequences of mutations in miRNA genes, we selected and functionally characterized 32 mutations located in different regions of the miRNA precursor. Using various approaches, we showed that mutations affect the precursor structure/stability, mature miRNA levels, precision of DROSHA/DICER1 cleavages (isomiR distribution), miRNA guide strand selection, miRNA target recognition, and miRNA silencing efficiency.

We also developed Whole-miRNome Sequencing (WMS), a highly effective, low-cost, and sample-saving approach for targeted sequencing of all (~2,000) human miRNA genes and 28 miRNA biogenesis genes. We used WMS to sequence ~600 samples, including ~300 cancer samples. In total, we identified 2,016 mutations, including 1,435 cancer somatic mutations, with 879 occurring in miRNA genes. The analysis identified several miRNA genes with functional enrichment of cancer mutations, including *MIR3928*, specifically mutated in basal cell carcinoma (BCC), indicating its potential role in this cancer. WMS also allowed the identification of multiple copy number alterations, hotspots of which often encompassed miRNA genes.

P39

Epitranscriptomic control of functional splicing through RNA cap methyltransferase CMTR1 directs mitochondrial dynamics during T cell activation

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T cell activation is a coordinated process involving increases in RNA transcription, processing and translation. RNA cap methyltransferase 1 (CMTR1) directs first nucleotide ribose 2'-O methylation (N1 2'-OMe) of RNA polymerase II transcripts and is induced following T cell receptor stimulation. N1 2'-OMe marks mRNA as self, thus avoiding recognition by anti-viral sensors/effectors such as RIG-I and IFIT. In addition to mRNA, N1 2'-OMe occurs on RNA pol II-transcribed small nuclear RNAs (snRNAs) and in particular, N1 2'-OMe of the U2 snRNA is required for spliceosomal E-complex formation and splicing reactions. During T cell activation, differential mRNA splicing controls transcript isoform selection, directing functionally distinct proteins to be expressed. Using transcriptomic approaches we identified a functional splicing module regulating mitochondrial dynamics in T cells by altering the isoforms of proteins controlling mitochondrial fission and fusion. Through epitranscriptomic regulation of snRNA and mRNA, CMTR1 directs this splicing module during T cell activation to promote the development of longer mitochondria with increased respiratory capacity. Thus, CMTR1 upregulation supports the energetic demands of T cell survival and immunological responses.

P40

A Plant-Specific Interaction Network Linking Spliceosome Components and Exon Junction Complex Assembly in *Arabidopsis*

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Pre-mRNA splicing is a key step in gene expression in eukaryotes. It is responsible for the diversity of mRNAs expressed from the genome, enriching the diversity of protein products and their functions. It is also one of the most important levels of plant response to environmental changes and stress – being unable to move, these organisms need to protect themselves at the molecular level. Therefore, studying the plant spliceosome is particularly relevant.

It is known that the composition of individual spliceosome complexes varies significantly across the tree of life. However, CWC22 protein is among the key spliceosome proteins, highly conserved and present even in greatly reduced spliceosomes. We have identified an Arabidopsis homolog of human CWC22. Our analyses indicate that, despite strong conservation within the two domains of this protein, plant and animal CWC22 proteins are evolutionarily distinct.

We identified also a homolog of another important human splicing factor – CWC21/SRRM2 – in Arabidopsis. As human CWC22 is known to interact with translation initiation factor EIF4A3,we included plant EIF43 in our analysis. The results of our studies using confocal microscopy, molecular biology techniques and structure prediction, indicate not only interaction between Arabidopsis CWC22 and CWC21 proteins, but also CWC22 – EIF4A3 interaction and, surprisingly, CWC21 – EIF4A3 interaction, which has not been shown previously for any organism.

Our findings reveal a novel interaction between CWC22, CWC21, and EIG4A3 in plants, suggesting that these splicing factors may cooperatively contribute to spliceosome activation and exon junction complex assembly, forming a regulatory module that is, on the one hand, evolutionarily conserved, but on the other, may exhibit plant-specific features reflecting unique aspects of plant RNA processing.

P41

The role of RNA metabolism factors in *Arabidopsis thaliana* in response to biotic stress

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In plants, defects in RNA processing and decay cause developmental problems, impaired hormonal signaling and altered resistance to abiotic and biotic stress. *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) is the most widely used bacteria to assess plant-pathogen interactions. To evaluate the contribution of mRNA capping, pre-mRNA splicing and mRNA decay to the regulation of plant immunity, we tested the response of mutants defective in these processes to *Pst* infection or treatment to its effectors, flg22 and elf18. Specifically, we used mutants in four different RNA metabolism factors: 1/ LSM1 involved in mRNA cytoplasmic decay, 2/ SMD3 and SMD1, core components of the spliceosomal complex, 3/ XRN4, a major cytoplasmic 5'-3' exoribonuclease, and 4/ DXO1 involved in the removal of non-canonical NAD+ cap (deNADding), mRNA cap biogenesis and possibly also cytoplasmic cotranslational quality control (CTRD). Our results show that while *lsm1*, *smd3b* and *smd1b* mutants have increased susceptibility to bacteria, *xrn4* and *dxo1* mutants exhibit enhanced resistance to *Pst*. Importantly, the resistant phenotype was also observed for transgenic dxo1 lines expressing DXO1(WT), DXO1(E394A/D396A) catalytic mutant, and DXO1(DN194) lacking the unstructured plant-specific N-terminal domain. Our genome-wide transcriptome analysis of *lsm1*, *smd3b*, and *dxo1* plants infected with *Pst*, verified by northern blot, revealed that the lack of these proteins deregulates defence against *Pst* DC3000 infection at the transcriptional level. In the case of mutants in factors involved in mRNA decay, *lsm1*, *xrn4* and *dxo1*, also mRNA stability of pathogenesis markers was altered, indicating regulation also at the posttranscriptional level. In addition, we also observed changes in callose deposition, the pattern of MAPK activation and the production of reactive oxygen species induced by flg22 and elf18. Taken together, our data strongly support the regulatory role of different RNA metabolism factors in plant immune response.

P42

The role of ERA1 in chlororibosome biogenesis and translation

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Chloroplast translation retains the fundamental features of its prokaryotic ancestor but has evolved to incorporate unique regulatory mechanisms essential for plant development and stress adaptation. The biogenesis of functional ribosomes depends on the coordinated action of ribosomal proteins, rRNA maturation, and specialized assembly factors. In this study, we identify ERA1, a plant homolog of the prokaryotic Era GTPase, as a crucial regulator of chlororibosome biogenesis and a key player in the chloroplast's response to environmental stress.

ERA1 localizes in chloroplast nucleoids, co-localizing with mTERF9, facilitating 16S rRNA processing and small ribosomal subunit (30S) assembly. Loss of ERA1 in *Arabidopsis thaliana* leads to the accumulation of immature rRNA precursors, mirroring the phenotype of bacterial *Δera* mutants. Reduced levels of Rps7, a key ribosomal protein, further indicate a delay in 30S subunit formation. Notably, these defects are most pronounced in young leaves, suggesting impaired translation kinetics during early chloroplast biogenesis. Moreover, *era1* mutants exhibit increased sensitivity to chloroplast translation inhibitors, confirming its crucial role in ribosome biogenesis. Genetic interactions with RBF1 and AtRimM, factors involved in 16S rRNA 3'-end maturation, further support the conserved function of ERA1.

The critical role of ERA1 is enhanced under environmental stress conditions, such as cold and high light – factors that primarily affect chloroplast gene expression. *Era1* mutants exhibit severe growth defects, reduced chlorophyll biosynthesis, and altered stress responses, reinforcing the link between impaired chloroplast translation and environmental adaptation.

Beyond its role in translation, ERA1 is essential for maintaining photosynthetic efficiency. *Era1* mutants display a significant reduction in PSI core proteins, whereas PSII components remain unaffected. This phenotype, resembling PSI-deficient mutants (*psaD*, *psaE*), is accompanied by constitutive LHCII phosphorylation via hyperactive STN7 kinase, likely driven by an enhanced reduced plastoquinone pool. Intriguingly, unlike PSI mutants, *era1* mutants maintain unaltered *STN7* transcript levels while exhibiting elevated STN7 protein abundance, suggesting altered protein turnover.

Ongoing investigations aim to decipher how ERA1 influences translation efficiency of specific chloroplast transcripts to elucidate the mechanistic basis of this regulatory interplay. Altogether,

our findings uncover an unknown link between chloroplast ribosome assembly, photosynthetic regulation and stress response, highlighting ERA1 as a central integrator of these processes.

P43

pyRBDome: A comprehensive computational platform for enhancing and interpreting RNA-binding proteome data

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By facilitating rapid transcriptome remodelling, RBPs enable cells to swiftly counteract unfavourable environments. We use the multi-drug-resistant Staphylococcus aureus as one of our model organisms. To understand how RBPs in S. aureus contribute to transcriptome remodelling, we conducted high-throughput proteomics experiments to uncover the S. aureus RNA-binding proteome under infection conditions. These experiments yielded surprising results, including the identification of cell wall proteins implicated in antibiotic resistance, which were not previously associated with RNA-binding but were experimentally verified as genuine RBPs. However, our data also raised concerns about the level of noise produced by these methods, particularly the number of false-positive hits.

To address this, we developed pyRBDome, a pipeline for enhancing RNA-binding proteome data in silico. It aligns the experimental results with RNA-binding site (RBS) predictions from distinct machine-learning tools and integrates high-resolution structural data when available. Its statistical evaluation of RBDome data enables quick identification of likely genuine RNA-binders in experimental datasets and amino acids that could bind RNA. Furthermore, by leveraging the pyRBDome results, we have enhanced the sensitivity and specificity of RBS detection through training new ensemble machine-learning (ML) models.

Surprisingly, pyRBDome analysis of a human RBDome dataset, compared with known structural data, revealed that although UV–cross-linked amino acids were more likely to contain predicted RBSs, they infrequently bind RNA in high-resolution structures. This underscores the limitations of structural data as benchmarks.

Using the spCas9-RNA complex as an example, we demonstrate that our ML model predictions substantially outperform the experimental cross-linking approach, positioning pyRBDome as a valuable alternative for experimental approaches when these are not applicable.

P44

Co-translational assembly of RNA polymerase I and III in yeast Saccharomyces cerevisiae

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RNA polymerases (RNAP) I and III are complex protein assemblies responsible for producing various RNA species. RNAPI and RNAPIII consist of 14 and 17 subunits respectively and share an assembly platform structure formed by four common subunits. RNAPI transcribes rRNA, whereas RNAPIII is involved in transcribing several non-coding RNAs, including tRNA, U6, and 5S rRNA. To date, it is understood that RNAPs are assembled in the cytoplasm before being transported as mature complexes to the nucleus. However, the exact biogenesis pathway of these complexes remains unclear. Understanding this process has become critical due to numerous mutations that lead to rare human diseases.

Co-translational assembly is a recently described phenomenon that takes place during the biogenesis of protein complexes. It involves the folding of individual subunits supported by a protein partner from the complex during the production of one or both proteins. This contrasts with the binding of fully folded proteins after translation.

Given the structural complexity of RNAPs, it is likely that their subunits require folding partners and undergo co-translational assembly. To test this hypothesis in yeast S. cerevisiae, we employed a modification of immunoprecipitation techniques, stabilizing elongating ribosomes using tagged polymerase subunits. Co-translational enrichment followed by high-throughput protein and RNA analysis revealed that several RNAPI and RNAPIII subunit mRNAs are enriched in samples with stalled translation.

This interaction is most likely facilitated by the Rbs1 protein, which has an N-terminal RNA binding domain and an unstructured C-terminal domain that may bind protein partners through multivalent interactions. Rbs1 interacts with mRNA of multiple RNAP subunits and protein subunits constituting RNAPI and RNAPIII. Furthermore, we demonstrate that the RNA binding SUZ domain is crucial for suppressing assembly defects in the Rpc128 mutant of RNAPIII. Our findings reveal the complexity of the RNAPI and RNAPIII assembly process, which is significant for both fundamental biology and understanding mechanisms of human diseases.

P45

Engineering circular RNAs for selective protein expression in dysfunctional endothelial cells.

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The success of the messenger RNA (mRNA)-based vaccines against COVID-19 proved the clinical potential of RNA technology for protein production in vivo. Circular RNA (circRNA) is a new class of single-stranded RNA with a closed-loop structure that improves its stability due to the lack of free ends necessary for degradation by exonucleases. In contrast to mRNA, circRNA is translated via Internal Ribosome Entry Site (IRES)-mediated translation that often relies on the assistance of factors known as Internal Ribosome Entry Site Trans-Acting Factors (ITAFs) which may vary in their expression depending on the cell type and cell state. IRES engineering could provide an avenue for tissue-specific expression by making circRNA the preferred RNA for the ribosome in a cell under stress conditions such as hypoxia and inflammation, relevant in high-medical-need diseases such as sepsis or cancer. Here, we investigated a library of IRES sequences for their ability to drive robust and specific circRNA translation. CircRNAs containing the 40 viral and eukaryotic/cellular IRES candidates and the Gaussia luciferase reporter gene were synthesised, purified and assessed in vitro. We found that Group IV viral IRES displays robust secreted luciferase activity in both human endothelial cells (ECs) (HUVEC, HPMVEC) at 24 h after transfection. This level of expression was followed by Group III and Group II viral IRES, with the weakest performance from Group I viral IRES. We could not detect any activity from eukaryotic/cellular IRES. This data was correlated with an in-silico analysis of the IRES candidates to predict potential ITAFs binding the circRNA, with Group IV viral IRES showing the highest number of interactions with RNA-Binding Proteins (RBPs). Moreover, IRES-containing circRNAs were delivered into both ECs under inflammatory and hypoxic conditions to study protein expression in comparison to its linear counterpart. LPS and TNF-α activation did not significantly affect the performance of the IRES-containing circRNAs in ECs. Furthermore, we observed differences in translation between circRNA and mRNA under hypoxia.

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Translation initiation at the 5'-polyadenylated and uncapped mRNAs from the yeast virus-like elements

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Linear cytoplasmic DNA plasmids pGKL1 and pGKL2, classified as virus-like elements (VLEs), have been found in the yeast Kluyveromyces lactis. VLEs encode a killer toxin and exhibit specific properties, including host independent replication and transcription. However, the mechanisms of transcription and translation of the VLE-specific genes have not yet been fully elucidated.

Transcripts of most of the VLE ORFs unusually possess 5' non-templated poly(A) leaders and lack a cap and polyadenylation at their 3' ends. It is thus puzzling how the VLEs transcripts are translated and what proteins play a role in translation initiation.

We created reporters based on the pGKL1 that express Renilla luciferase-encoding mRNAs with different 5' ends including uncapped and 5-polyadenylated mRNAs. The aim is to use these reporters in cells lacking selected translation initiation factors. We attempt to identify translation initiation factors that are important for translation of uncapped and 5' polyadenylated mRNAs using the TREX method (targeted RNase H-mediated extraction of crosslinked RBPs; https://doi.org/10.1038/s41592-024-02181-1).

We will demonstrate first results obtained using the aforementioned experimental approaches in the analysis of translation initiation at the yeast VLE mRNAs.

Acknowledgement

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Effects of genetic ablation and pharmacological inhibition of HuR on gene expression, iron metabolism, and hormone levels

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HuR/ELAV1, a ubiquitous RNA-binding protein, belongs to the RNA-binding protein family and is crucial for stabilizing and regulating the translation of various mRNA targets, influencing gene expression. Elevated HuR levels are associated with multiple disorders, including cancer and neurodegenerative diseases. Despite the identification of small molecule inhibitors targeting HuR, their detailed characterization remains limited. Recently, Eltrombopag, an FDA-approved drug for immune thrombocytopenic purpura and chemotherapy-induced thrombocytopenia, emerged as a potential HuR inhibitor. However, the specific molecular pathways influenced by both HuR and Eltrombopag are not fully understood.

Our study demonstrates that Eltrombopag operates via HuR inhibition, affecting gene expression regulation at the posttranscriptional level. We show that both HuR knockout and Eltrombopag treatment modulate iron metabolism by decreasing ferritin heavy chain (FTH1) and light chain (FTL) synthesis while increasing the expression of iron-regulatory protein 2 (IRP2), a key regulator of ferritin translation. Additionally, HuR inhibition reduces the levels of glycoprotein hormones, alpha polypeptide (CGA), a marker associated with hormone-induced tumors, suggesting a potential use of Eltrombopag in treatment of cancers overexpressing CGA. We observed that the main of control is manifested at the level of translation inhibition, with proteasome-mediated regulation also playing an important role.

These findings uncover novel posttranscriptional mechanisms governed by HuR and its inhibitor, elucidating

pathways relevant to HuR-mediated regulation and molecular therapies aimed at targeting this protein.

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Silymarin and Hepatic Health: Insights from Pig *(Sus scrofa)* and Human *(Homo sapiens)*Research

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Silybum marianum, commonly known as milk thistle, has long been recognized for its hepatoprotective and gastrointestinal benefits. Its active compound complex, silymarin, comprises flavonolignans such as silybin, isosilybin, silychristin, dihydrosilybin, and silydianin, which possess the capacity to modulate gene expression pathways involved in detoxification and cellular stress responses. Given the herb's ability to counteract the detrimental effects of poor dietary habits, its therapeutic relevance continues to gain traction in both human and veterinary medicine.

This study aimed to evaluate the hepatoprotective effects of S. marianum using a porcine model, given the physiological and genetic similarities between pigs and humans. Young pigs (n=154), noted for their immunological responsiveness, were selected to assess the systemic and hepatic impact of silymarin. Following slaughter, comprehensive organ and tissue sampling was conducted, including liver, kidney, heart, pancreas, spleen, pituitary gland, stomach, intestinal segments (duodenum, jejunum, ileum, colon, cecum), and blood serum (collected using heparin and EDTA). Pre- and post-mortem blood samples were obtained via venipuncture from the external jugular vein.

Hematological and biochemical analyses were carried out at the Municipal Hospital Laboratory in Olsztyn. Parameters included complete blood count indices (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, NEUT, LYM, MONO, EOS, BASO), immunoglobulin profiling (IgA, IgE, IgM, IgG), and metabolic markers (TC, HDL, LDL, TG, GLU) alongside liver function tests (ALT, AST, ALP, BIL,

GGTP).
Findings from this study contribute to the mechanistic understanding of silymarin's effects on hepatic health, particularly through its influence on metabolic regulation and immune responses. Moreover, transcriptomic analysis is underway to further elucidate gene expression changes associated with silymarin administration. The use of the pig model not only enhances the translational relevance of these findings but also supports the exploration of silymarin as a complementary strategy in the prevention and management of liver disorders.

Acknowledgment

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Characterizing the role of LARP6C proteins in pollen heat stress resilience of *Arabidopsis thaliana* and tomato.

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Pollen grains are highly sensitive to elevated temperatures, which threatens fertilization and crop productivity under climate change. The development of the pollen and its fertilization heavily rely on the post-transcriptional regulation, which is in part achieved by RNA binding proteins (RBPs). Among them, LARP6C, a pollen-specific RBP belonging to the family of LA proteins, has been shown to form dynamic cytoplasmic granules in the mature pollen and store translationally silent mRNAs in Arabidopsis. It's also been shown that they facilitate rapid translational reprogramming soon after transitioning from mature to hydrated pollen. Interestingly, LARP6C granules partially co-localize with stress granules (SGs), PABPs suggesting a potential role in stress adaptation.

Physiological studies in Arabidopsis have shown that *larp6c* mutants exhibit delayed germination and fail to progress through key developmental stages. Based on its interaction with PABPs and formation of granules in mature pollen, we hypothesize that LARP6C may play a vital role in protecting critical mRNAs from degradation during heat stress, similar to SGs in somatic cells. However, the role of LARP6C in pollen thermotolerance and its functional conservation in crops like tomato remains unexplored. Therefore, this study aims to (1) elucidate the physiological and molecular function of pollen-expressed LARP6 proteins in *Solanum lycopersicum* in two extreme thermotolerant varieties i.e. heat sensitive and heat resistant (Heinz and Tamaulipas respectively), (2) characterize the expression and subcellular localization of LARP6C under normal and heat stress conditions; in both Tomato and Arabidopsis, (3) evaluate how specific LARP6C protein domains (PAM, La-module, LSA) contribute to pollen tube development and male fertility, (4) investigate the functional shift of LARP6C during the transition from dry to hydrated pollen.

Using CRISPR Cas 9-generated knock-out mutants, domain-deletion constructs, and fluorescent reporters, we assess the impact of LARP6C on pollen performance under heat stress by several assays for both physiological, subcellular and molecular roles of LARP6C under normal and heat stress conditions. Understanding this mechanism could reveal how pollen sustains fertility under adverse heat conditions and strategies for improving thermotolerance in crops.

P50

The role of SERRATE C-terminal fragment in microRNA biogenesis.

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MicroRNA (miRNA) is a group of short ribonucleic acids, which are the final products of non-protein-coding MIR genes. MiRNA molecules are involved in the broad spectrum of post-transcriptional regulation of genes expression and proteins synthesis. In the process of miRNA biogenesis, a protein complex called the “microprocessor” plays a crucial role. Among the core components of this complex in plants, we can distinguish the Dicer-like endonuclease 1 (DCL1), the double-stranded RNA-binding protein – Hyponastic leaves 1 (HYL1) and the protein with the C2H2 “zinc finger” domain – SERRATE (SE). The SERRATE (SE) protein is involved in almost all stages of miRNA biogenesis. Research conducted on SE shows its significant impact in the processes of transcription regulation, splicing and RNA degradation. During many years of research on SE, 4 mutants were described: se-1, se-2, se-3, se-4. In the se-1 mutant, a deletion of 7 nucleotides was introduced at the 3' end of the gene, depriving the final protein of 27 highly conserved amino acids at its C-terminus. Even though the deletion is introduced at the C-terminus of the protein, a highly pleiotropic phenotype of the se-1 mutant was noticed. This phenomenon is interesting because the deletion covers part of the unstructured region, which goes significantly beyond the so-called “protein core”. The pleiotropic phenotype of the se-1 mutant combined with the knowledge of the high conservation level of the C-terminus of SE indicates a potentially important influence of the above-mentioned region in the interaction of SE with other proteins. To determine the role of the c-terminus of the SE protein, different length variants (27, 97 and 177aa) of the above-mentioned sequence were combined with the GFP protein to determine the interactions between the proposed sequences and other proteins occurring in the plant cell. Identification of proteins has been proceed using coimmunoprecipitation assay. Moreover, the SE protein produced by the se-1 mutant, which in turn was fused with the FLAG tag has also been used in this study. This experimental set up can give us valuable information about role of c-end terminus of SE protein in interaction among proteins and microRNA biogenesis.

P51

Exploring Potential Roles of human PNPase Beyond RNA Degradation

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Human PNPase is an evolutionarily conserved exoribonuclease associated with mitochondrial homeostasis, and RNA metabolism including turnover, processing, and trafficking. Dysfunction or mutations in human PNPase lead to oxidative phosphorylation deficiency, Leigh syndrome, delayed myelination, hereditary hearing loss, and mitochondrial disorders. hPNPase resides throughout mitochondria; it forms a degradosome complex with helicase SUV3 in the mitochondrial matrix, where it catalyzes RNA degradation via phosphorolysis. Despite of its significant role in mitochondrial and human health, the mechanism of action of PNPase, especially its role in the mitochondrial intermembrane space, is poorly understood
Our research hypothesis explores the function of intermembrane space localized hPNPase. I use Microscale thermophoresis, pulldown assays, and electron cryo-microscopy to study human PNPase and its interaction with RNA, and to determine whether and how the human enzyme can engage RNA in non-degradative mode. This hypothesis stems from the discovery of a novel role of bacterial PNPase, a close homologue of the human enzyme, which can be re-programmed to switch between RNA degradation and protection. The findings from our research have broader implications for understanding mitochondrial biology and may open avenues for targeted therapeutic strategies to address mitochondrial diseases associated with PNPase dysfunction and mutations.

P52

T4ph-Driven Definition of RNAPII Termination Regions

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Transcription termination marks the precise end of gene transcription, ensuring that RNA polymerase II properly disengages and the nascent RNA is released from the DNA template. Accurate mapping of transcription termination zones is critical for understanding gene regulation, RNA processing, and genome function. While the start of transcription is precisely defined at transcription start sites (TSS), termination remains far less annotated, often extending thousands of nucleotides beyond the polyadenylation site (PAS). This ambiguity limits our ability to fully interpret transcript isoforms, antisense regulation, and read-through transcription events implicated in disease.

Recent studies of Kopczyńska et al. (NAR 2025) highlight threonine 4 phosphorylation (T4ph) of the RNA polymerase II C-terminal domain (CTD) as a robust and biologically distinct marker of transcription termination. T4ph accumulates downstream of PASs on actively transcribed protein-coding genes, marking the zone where Pol II pauses prior to release. Unlike traditional elongation or initiation-associated CTD marks (such as Ser2P or Ser5P), T4ph is specifically associated with the late stages of transcription and aligns with decreased nascent RNA synthesis and Pol II disengagement.

Despite its potential, T4ph remains underutilized due to challenges in signal detection. Its broad, fragmented signal profile is poorly captured by peak-calling algorithms such as MACS2, which were developed for sharp, localized enrichments. Furthermore, T4ph detection relies on methods like mNET-seq, which provide strand-specific and single nucleotide resolution data, that current tools are not well-optimized to handle.

To advance our understanding of RNA biology, there is a pressing need for improved computational strategies that can define T4ph-enriched termination windows with greater precision. This will allow researchers to better distinguish between productive and premature termination events and uncover new regulatory layers controlling RNA fate.

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P53

Translation in ALS: The Role of FUS in rRNA Processing and translation efficiency

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder primarily affecting motor neurons. One of the most aggressive familial forms of ALS is caused by the P525L mutation in the RNA-binding protein FUS, which mislocalizes to the cytoplasm, forms toxic aggregates, and impairs key RNA metabolic processes. Emerging evidence suggests that FUS also regulates small nucleolar RNAs (snoRNAs), which guide site-specific modifications of ribosomal RNA (rRNA), including 2'-O-methylation and pseudouridylation. These modifications are crucial for proper rRNA processing and ribosome biogenesis. Their disruption may alter ribosome composition and function, contributing to translation dysregulation observed in ALS.

In this study, we investigated translational disturbances and rRNA processing defects in a human cellular model of ALS-FUS. Using induced pluripotent stem cells (iPSCs) derived from ALS patient fibroblasts carrying the P525L mutation and their isogenic wild-type controls, we generated neuronal progenitor cells (NPCs) and compared translational activity and rRNA maturation.

Translational efficiency was measured using the SUnSET assay, which enable to analyse changes in global translation in FUS mutant cells compared to wild-type cells. In parallel, we performed RT-qPCR to quantify key steps of rRNA maturation, targeting both precursor (45S, 47S) and mature (5.8S, 18S, 28S) rRNAs. The analysis let us see any disturbances in levels of early precursors and mature rRNA forms in ALS-FUS NPCs, indicating disrupted ribosome biogenesis.

Our findings will support the hypothesis that FUS mutations disrupt snoRNA-guided rRNA maturation, which in turn may affect ribosome integrity and protein synthesis in neuronal cells. Together, the observed defects in rRNA biogenesis and translational capacity in ALS-FUS NPCs will provide further insight into the molecular mechanisms linking RNA metabolism and neurodegeneration.

P54

Targeting RNA molecules with synthetic ligands – structural investigation focused on neurodegenerative disorders caused by expanded repeats.

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The presented work is a part of our crystallographic studies focused on structural analysis of interactions between disease related RNA and synthetic molecules. Although the recent progress in high-throughput screening of small molecule libraries resulted in discovery of a number of drug-like compounds their further improvement requires determination of three-dimensional structures unravelling the details of RNA-ligand interactions.

We will present crystallographic studies of complexes of small ligands and RNAs associated with neurodegenerative disorders called TREDs (Trinucleotide Repeat Expansion Disorders). The abnormal expansion of repeated sequences located within certain genes results in mutated mRNAs gaining pathogenic properties. The specific binding of small molecules to mutated RNA can block pathological pathways preventing disease progression. We analysed a series of small molecules recognizing unique pattern of nucleobases engaged in non-canonical pairing or located in single stranded regions of repeated RNA sequences. Structures of the complexes allowed detailed characterization of interactions between the ligand and RNA indicating how small compounds can be improved for future biomedical studies.

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Deciphering the role of epitranscriptomic marks in double-stranded RNA sensing by human and avian MDA5 receptor

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RNA viruses, including Influenza A virus (IAV), pose a serious epidemic threat due to their high mutation rate and zoonotic potential. Domestic poultry – particularly chickens and ducks – serve as semi-natural reservoirs for IAV and other viruses such as Newcastle Disease Virus and Infectious Bronchitis Virus, with intensive farming promoting both viral spread and interspecies transmission. Chickens are notably more susceptible to IAV than ducks, likely due to differences in innate immune receptors that detect viral double-stranded RNA (dsRNA). Long dsRNAs, produced as a viral replication intermediate, act as potent pathogen-associated molecular patterns, typically recognized by RIG-I-like receptors such as RIG-I and MDA5. While ducks and humans express both receptors, chickens lack RIG-I depending solely on MDA5. To evade immune detection, viral RNAs are decorated with internal chemical modifications (epitranscriptomic marks) that mimic those present in endogenous eukaryotic transcripts such as 5-methylcytosine (m5C), N6-methyladenosine (m6A), or pseudouridine (Ψ). Although unstudied, these modifications are likely to modulate the efficiency of dsRNA detection by MDA5. **The aim of this project is to investigate how specific nucleotide modifications in viral long dsRNA affect MDA5-mediated recognition.** As a model simulating viral intermediates, we will prepare dsRNA molecules containing selected epitranscriptomic tags (e.g., m5C, m6A, Ψ). Using a combined in vitro and in cellulo approach, we will assess whether these modifications alter dsRNA recognition by MDA5. First, dsRNAs will be used in affinity assays with recombinant human, chicken, and duck MDA5. The stability of MDA5-dsRNA complexes will be analyzed by gel-shift assay. We will also investigate the oligomerization pattern of MDA5 protein on dsRNA variants using Cryo-EM. In parallel, CRISPR-Cas9 will be used to generate DDX58 (RIG-I) and IFIH1 (MDA5) knockouts in duck fibroblast. Subsequently, these lines will be used to evaluate immunogenicity of modified dsRNAs in cellulo. Moreover, UV-Vis melting analysis will be used to assess the physical stability of each dsRNA variant and its impact on immunogenicity. This study will provide insight into how dsRNA modifications influence innate immune sensing and contribute to host-specific differences in susceptibility to viral infections.

P56

From Transcriptomic Waves to Phenotypic Outcomes: A Newly Identified Arabidopsis Protein Complex Related To Splicing, Regulating Salinity Stress Response and Embryonic Development

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Splicing regulation plays a crucial role in plant development and stress adaptation. Here, we report the discovery of a novel *Arabidopsis thaliana* protein complex composed of six subunits: CWC22, CWC21, CYP95, CYP63, SC35, and SR34a, with CWC22 as a highly evolutionarily conserved core component. Protein interactions within this protein assembly were confirmed through yeast two-hybrid assays, co-localization studies, and bimolecular fluorescence complementation, with additional binding validation for CWC22-CWC21 *via* MicroScale Thermophoresis and pull-down assays on purified proteins.

CWC22, a known splicing factor, features MIF4G and MA3 domains connected by a flexible linker and flanked by low-complexity N- and C-terminal sequences. Our Fluorescence Recovery After Photobleaching (FRAP) experiments suggest that CWC22 forms dynamic nuclear condensates *in vivo*. While the CWC22-CWC21 interaction is well-documented in humans, the conservation of CWC21 across species is lower. Phylogenetic analyses and experimental validation confirm At3g49601 as the Arabidopsis homolog of CWC21.

Phenotypic analysis of Arabidopsis mutants for three subunits—CWC22, CWC21, and CYP95—revealed key roles in embryogenesis and stress response. About 25% of seeds in heterozygous *cwc22* siliques cease development around six days post-pollination. Seed genotyping showed that seeds exhibiting embryonic lethality are homozygous for *cwc22*. Microscopic analysis (Nomarski DIC) combined with single-seed RNA sequencing provided insights into the stage and molecular mechanisms of embryo arrest in *cwc22 -/-*. Similar analyses were conducted on seeds from *cwc21* and *cyp95* mutants, revealing no morphological changes but highlighting significant molecular differences compared to wild-type seeds.

In salinity stress assays (150mM NaCl), wild-type (*Col-0*) seeds germinated at ~70%, whereas *cwc21*, *cwc22+/-*, and *cyp95* mutant seeds showed significantly higher germination rates (82.1%, 79.5%, and 96.4%, respectively). RNA-seq analysis under control and salt stress conditions on hydroponically grown plants further validated the complex's role in transcriptomic regulation. Under control conditions, differentially expressed genes (DEGs) numbered 485 in *cwc21*, 880 in *cwc22+/-*, and 1089 in *cyp95*, with 87 shared DEGs. After 90 minutes of salt stress, these numbers

increased to 839, 1569, and 1132 DEGs, respectively, with 178 shared genes exhibiting conserved expression directionality across all mutants.

These findings suggest that this newly identified splicing-related protein assembly plays a crucial role in transcriptome remodeling, embryonic development, and salinity stress adaptation, pointing to its significance in plant resilience mechanisms.

P57

Identification of MBNL1 protein interactome and its role in regulating MBNL1-dependent alternative splicing

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Muscleblind-like proteins (MBNLs) are tissue-specific RNA-binding proteins (RBPs) that are responsible for RNA metabolism, including alternative splicing (AS). AS is a process in the nucleus by which different mature mRNAs are generated from one gene, synthesizing different functional proteins. The examples of the MBNL-dependent AS events were comprehensively described in the literature, and many of them cover the pathogenesis of myotonic dystrophy type 1 (DM1). DM1 is an uncurable, autosomal dominant muscle disease, characterized by *i.a.* progressive muscle weakness, myotonia, and heart issues. It is caused by trinucleotide expansions (CTG^{exp}), over 50 repetitions, within the 3'UTR region of the *Dystrophia Myotonica Protein Kinase* (*DMPK*) gene. The forming toxic RNA has the potential to sequester some proteins, and the most important ones are MBNLs. The consequence of sequestration is the disruption of the AS, what leads to the disease phenotype. Until now, little is known about MBNL's interactions with other proteins in both physiological and pathological conditions.

This project aimed to study interactions between MBNL1 and its potential partners and understand the mechanism of those associations. The results obtained previously by high-resolution mass spectrometry (HRMS) were utilized for the identification of the possible MBNL1 protein partners. Then, the BioID2 pull-down demonstrated that SFPQ is located close to MBNL1 in a cell, suggesting their potential interactions, while applying the GFP-trap pull-down enabled to prove that the N-terminus of MBNL1 protein is crucial for interaction with SFPQ. Further analyses showed that MBNL1-SFPQ binding is partly RNA-dependent. Later, the mechanism of MBNL1-SFPQ interaction was examined by studying the MBNL-dependent AS regulation. In this way, the genes that AS events are potentially co-regulated by MBNL1 and SFPQ were identified.

This research could pave the way for a deeper understanding of MBNL1 and its partners' roles in their physiological environment but also may have implications for future studies, including those linked to neurological diseases, including DM1.

P58

Downregulation of CWC22 splicing factor leads to global changes in gene expression and splicing stimulating renal cell carcinoma cell migration

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Dysregulation of alternative splicing (AS), observed in cancer, disrupts key genes involved in tumorigenesis, often due to aberrant splicing factor expression. Renal cell carcinoma (RCC), the most prevalent kidney malignancy, exhibits significant AS alterations, with underlying causes remaining largely unknown.

Our analysis revealed that the expression of the splicing factor CWC22 is significantly reduced in advanced stages of renal cell carcinoma (RCC), and its diminished expression is associated with unfavorable patient survival outcomes

CWC22 plays a critical role in stabilizing 5' exons prior to the first splicing step and positioning the exon junction complex (EJC) on mRNA, thereby influencing splicing, mRNA export, nonsense-mediated decay and translation.

To investigate the functional significance of CWC22 in RCC, we silenced its expression using siRNA in three cell lines: 786-O and A498 (derived from primary tumors), and Caki-1 (derived from a metastatic lesion), which together represent the heterogeneity of clear cell RCC (ccRCC).

Microarray analysis showed that CWC22 silencing led to substantial transcriptomic changes, with differentially expressed genes (DEGs) numbering 3321, 2715, and 2274 in 786-O, A498, and Caki-1 cells, respectively. Among 429 common DEGs, we identified oncogenic drivers such as HIF1A and genes involved in mRNA processing (QKI, SF3B3, SKP2, and MERCKS). Ingenuity Pathway Analysis (IPA) of DEGs revealed enhanced tumor cell migration and motility in all cases, while proliferation was mildly or not activated in 786-O and Caki-1, and inhibited in A498.

Even more profound alternations were observed in splicing, over 5000 transcripts underwent AS across all models following CWC22 depletion, with pathway enrichment analysis implicating Rho GTPase signaling and cell cycle regulation. Transcriptomic shifts and AS events in 786-O cells were further characterized via direct RNA sequencing using Nanopore technology.

Cellular functional assays demonstrated that CWC22 downregulation significantly increased the motility of 786-O cancer cells, while moderately reducing proliferation and viability. This findings are consistent with the results of the transcriptomic analysis.

Our findings identify CWC22 as a crucial regulator of both transcription and AS in RCC cells. IPA and enrichment analyses revealed alterations in critical cellular processes, linking the loss of CWC22 to tumor progression through enhanced cell migration and its potential role in metastasis.

P59

Improving amiRNA design to effectively target mutant CAG repeats in huntingtin-lowering therapy

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Huntington's Disease (HD) is a fatal neurodegenerative disorder caused by an abnormal CAG repeat expansion in the huntingtin (HTT) gene. This mutation leads to the production of a toxic form of huntingtin protein (mutant HTT). To selectively target the mutant allele, we previously designed an allele-selective artificial microRNA (amiRNA) construct, which reduces mutant HTT levels by approximately 50% in the YAC128 mouse model of HD, without affecting wild-type HTT expression. These findings represent a significant step towards achieving allele-selective silencing in HD. However, to enhance this approach's efficacy and safety profile, we aim to improve the amiRNA's efficiency to achieve comparable silencing effects at lower doses.

Our current project focuses on optimizing this amiRNA construct through two main strategies:

(1) modifications near the specific Drosha and Dicer processing sites within the amiRNA sequence, aimed at enhancing its biogenesis and effectiveness, and

(2) a combinatorial strategy involving tandem amiRNA constructs, which are expected to have an additive or synergistic effect on HTT silencing. Tandem amiRNAs offer potential advantages, as multiple targeting sequences within a single construct could reduce the required dosage for effective mutant HTT knockdown.

Preliminary data from cell studies indicate that both approaches significantly improve the efficiency of mutant HTT silencing at lower doses than the basic amiRNA construct. Notably, the amiRNA variant containing sequence motifs that enhance processing precision emerged as the most potent construct in our assays. One of the tandem amiRNA constructs also exhibited a comparable level of silencing efficiency, suggesting that tandem amiRNAs may be a promising avenue for HD therapy, particularly by reducing dosage requirements and thereby potentially minimizing off-target effects

P60

IDIOSYNCRASY OF ANTISENSE OLIGONUCLEOTIDE TARGETING PROTEINCODING GENE EMBEDDED WITH NON-CODING RNA IN VIVO

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ASOs (antisense oligonucleotides) are a promising therapeutic approach for suppression, induction of gene expression or the correction of aberrant splicing. Addressing whether ASOs targeting genes embedded with intronic noncoding RNAs (ncRNAs) affect the expression and function of intronic ncRNAs is of importance to the success of ASOs in clinical trials. While studying the development of the zebrafish posterior pituitary (neurohypophysis), an important neuroendocrine interface, we observed that an ASO targeting the splice site, in contrast to the one targeting the translation site of the gene *slit3*, disrupts neurohypophyseal axonal morphogenesis. In addition to altered *slit3* splicing, we also observed an increase in the expression of *slit3* and *slit3* intron-embedded primary *mir218a-1* transcripts. The ASO-induced phenotype was not observed when mature *mir218a-1* was blocked by an ASO or in *mir218a-1-/-* mutants. In addition, we also found that previously reported phenotypes due to ASOs targeting the splice site of *pank2* and *dnm2a* were partially rescued when the mature *mir103* and *mir199-5p* embedded in their introns, respectively, were blocked by ASOs. Our observation that ASOs targeting splice sites can affect intronic microRNA expression and function warrants further validation for other classes of ncRNAs. In addition, the idiosyncratic phenotypes when using translation and splice-blocking ASOs can be potentially used as a marker to identify the role of intronic ncRNAs.

P61

Characterization of the IFIT proteins role in innate immunity

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IFITs (interferon-induced proteins with tetratricopeptide repeats) are a family of RNA-binding proteins, known for their role in the innate immune responses in vertebrates. Their expression is induced in response to interferon, viral infection, or recognition of PAMP (pathogen-associated molecular pattern). In humans, the IFIT family consists of five paralogues – IFIT1, IFIT1B, IFIT2, IFIT3 and IFIT5. Among IFIT proteins, only IFIT5 occurs as a monomer, while IFIT1, IFIT2 and IFIT3 form homo- or hetero-complexes with themselves. Antiviral properties of IFITs originate from their ability to discriminate foreign RNA, based on its molecular patterns. The molecular patterns recognized by IFITs include cap0 (IFIT1) or AU-rich sequences (IFIT2), that may resemble AU-Rich Elements (AREs).

In our research, we investigate the antiviral and RNA regulatory functions of IFIT proteins. To identify RNA molecular partners of IFIT2, we co-expressed and purified a recombinant IFIT2/3 heterodimer, which we employed as bait in RNA pull-down assays. Interestingly, this approach revealed selective enrichment of IFIT mRNAs, suggesting a potential autoregulatory mechanism whereby IFIT proteins may bind and modulate the expression of their own transcripts. To explore the role of IFIT proteins in the regulation of host mRNAs, we optimized the iCLIP2 protocol for 3xFLAG-tagged IFIT1 in A549 cells. This method enables high-resolution mapping of RNA–protein interactions and lays the foundation for transcriptome-wide identification of IFIT1-bound RNAs under physiological or stimulated conditions. In parallel, we investigated the role of IFIT proteins in the context of viral infection. Using an A549 cell line with a knockout of the IFIT3 gene, we observed a significant increase in Sindbis virus (SINV) replication compared to wild-type cells. These findings uncover a previously unrecognized antiviral function of IFIT3.

P62

The regulatory function of *miR-27b-5p* in the head and neck squamous cell carcinoma biology

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Non-coding RNAs are among the most crucial and universal regulators of cell biology, with the potential to become future biomarkers or therapeutic targets in personalized oncology treatment. Patients developing head and neck squamous cell carcinoma (HNSCC), one of the most prevalent, heterogeneous, and therapeutically challenging cancers worldwide, could particularly benefit from this novel approach. Recently, many reports indicated that microRNAs are frequently aberrantly expressed and implicated in processes pivotal for cancer development, e.g., miR-27b, a known epithelial-to-mesenchymal transition (EMT) regulator.

Expression and patients' clinicopathological data obtained during The Cancer Genome Atlas (TCGA) project were downloaded from the University of California Santa Cruz and the cBioPortal databases to study survival curves, gene enrichment, deconvolution results, and potential targets of both miR-27b mature strands using online tools. Subsequently, two cell lines – FaDu and SCC-040 – were modified with a miR-27b or control plasmid and subjected to functional tests, assessment of their response to standard oncology treatment, and transcriptome analysis using RNA-seq. Expression and protein levels of chosen potential targets were assessed via qRT-PCR and/or the Western blot method.

In TCGA samples, *miR-27b-5p* had a higher expression level in cancer compared to the non-pathologically changed margin. Analyses of clinicopathological parameters determined that an increased level of the 5' strand was associated with better overall survival, more favorable outcomes, and immunological profiles. The elevated *miR-27b-5p* level was linked with reduced proliferation and negative regulation of the cell cycle, and its' decreased level was associated with upregulation of genes involved in processes promoting cancer growth, e.g., EMT and TGF-β signaling pathway, based on both – TCGA data and cell line experiments. Lastly, models with induced miRNA overexpression were more sensitive to the cisplatin therapy and, interestingly, had a worse response to applied irradiation and administered doxorubicin.

The above emphasizes the significant impact of *miR-27b-5p* on cancer biology. Further research exploring its vast regulatory potential might provide a better understanding of HNSCC and new therapeutic approaches leading to improvement of the quality and life expectancy of cancer patients.

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P63

The Spliceosome as an Architect of Life at the RNA Level: AtCWC22 and CYP95 as Key Players in the Life and Death of *Arabidopsis* Seeds

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Proper development and maturation of angiosperm seeds require tightly regulated transcriptomic, metabolic and epigenetic programs. In this study, we focused on the role of the spliceosomal proteins: AtCWC22 – the Arabidopsis ortholog of human CWC22 – and its interactors: AtCWC21 and cyclophilin CYP95. In the complex process of eukaryotic gene expression, transcription and pre-mRNA splicing are critical steps that contribute to transcriptome diversity and functional proteome complexity. CWC22, a well-characterized general splicing factor required for spliceosome assembly and pre-mRNA splicing in human cells. Our analysis indicates that its Arabidopsis ortholog plays a crucial role in plant development.. The absence of homozygous mutants, together with the occurrence of deformed seeds in the siliques of heterozygous plants, supports its essential function during embryogenesis.

Single-seed RNA-seq analysis revealed that, under normal developmental conditions in wild-type *Arabidopsis* (Col-0), numerous photosynthesis-related genes are transcriptionally down-regulated between 5 and 6 days after pollination (DAP), while ribosomal genes are activated. In contrast, *cyp95* mutants display a disturbance in this regulatory program, characterized by abnormal upregulation of photosynthetic genes, no significant change in ribosomal genes transcription and repression of stress response genes, which likely compromise seed longevity. This is supported by a reduced germination rate observed in *cyp95* seeds after prolonged storage.

Transcriptomic analysis suggests that CYP95 – unlike AtCWC22 and AtCWC21 – may regulate seed maturation through mechanisms involving plastid gene expression. At the same time, 5DAP and 6DAP seeds of *cwc21* and *cyp95* mutants show similar transcriptomic profiles, indicating their possible cooperation within the splicing-related and other regulatory pathways. Notably, the single seed transcriptome of *atcwc22+/-* mutant suggests a distinct role in maintaining viability and proper embryogenesis.

Our results indicate that spliceosome components, beyond their well-established functions in pre-mRNA splicing, may also play important roles in transcriptional regulation. Disruptions in their expression levels impair seed developmental control, and appears to affect both dormancy and longevity. These findings highlight spliceosomal factors as potential targets for seed biotechnology and contribute to a deeper understanding of the molecular regulation of seed development.

P64

Epigenetic control of transcriptional landscape during keratinocyte differentiation

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Mammalian epidermis is a stratified, self-renewing epithelium consisting of several layers of keratinocytes crucial for survival. As keratinocyte undergo differentiation they move upwards, loose their proliferative capacity while upregulating genes involved in epidermal barrier formation to finally undergo skin-specific version of programmed cell death: corneoptosis, which is indispensable for the formation of fully functional skin. Those dramatic changes are driven by transcriptional program tightly controlled by several epigenetic pathways and affected by remarkable changes in 3D genome architecture.

Our results show how changes in nuclear compartments set by repressive Polycomb complexes PRC1 and PRC2, as well as nuclear lamina and nucleoli contribute to establishment of the transcriptional profiles of distinct subpopulations of keratinocytes. As the differentiation progresses the amount of chromatin bound to nucleoli and nuclear lamina increases, similarly to the global level of Polycomb repression. Those changes, besides directly affecting transcription, work also in more nuanced way by changing the chromatin folding and rewiring of promoter-enhancer interactions. Intriguingly, most keratinocyte-specific structural genes are located within three genomic loci: EDC, Ker I and Ker II. EDC spanning 3Mbp is inflicted in several diseases including psoriasis and atopic dermatitis. We show that despite high activity part of the EDC is associated with nuclear lamina which seem to fine tune its gene expression together with changes in the activity of PRC1/2.

To sum up our results coming from set of genomic experiments provide novel insight into the role of the PRC1/2 complexes as well as changes in 3D nuclear architecture in the control of gene expression during establishment of epidermal barrier.

P65

U35K of U11/ U12snRNP is indispensable for survival in heat stress of *Arabidopsis thaliana* plants

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Minor U11/U12snRNP is a ribonucleoprotein complex built of at least six proteins and U11 and U12 snRNAs. It participates in splicing of minor introns containing specific 5' splice site and branch site sequences. Null mutants of *A. thaliana u11snRNP-35k* (*35k*) and *u11snRNP-48k* (*48k*) genes exhibit flowering retardation when compared to wild type plants. However, in heat stress (30°C) *35k* mutant is not able to survive while *48k* does not show defects in plant development. Anatomical analysis of SAM (Shoot Apical Meristem) revealed that in the case of *35k* mutant SAM degenerates in both vegetative and generative photoperiods, resulting in strong developmental aberrations leading to plant death. Transcriptomic analyses performed for 12 day-old-seedlings in WT plants, *35k* and *48k* mutants allowed us to identify 142 genes containing U12 introns (out of 261 bioinformatically identified) that showed statistically significant differences in expression level between control and high temperature conditions. Out of these 142 genes 97 U12 introns (67%) showed intron retention (IR) in *35k* mutant at high temperature.

Interestingly, we found that under heat stress, heat shock protein (HSP) 101, essential for surviving severe heat stress, is induced in WT plants and in the *48k* mutant while not in the *35k* mutant. Analysis of transcriptomic data revealed, however, that HSP101 mRNA level is specifically upregulated in *35k* mutant at high temperature. Thus at least in the case of HSP101 it seems that the effect of the lack of HSP101 protein upregulation may result from translational inhibition or protein degradation processes. In addition, time-course experiment revealed that the important switch at sensing high temperature by *35k* mutant plants occurs at early stage of plant development and besides HSP101 protein may also involved SAM establishment and maintenance genes such *CLAVATA* (*CLV*) and *WUSCHEL* (*WUS*) as well as RISC-related *ARGONAUTE 10* gene (*AGO10*). The possible explanation of disturbed heat-stress response in Arabidopsis will be discussed.

P66

Novel regulatory elements at the 5' end of the MBNL1 locus with potential impact on MBNL1 expression

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Muscleblind-like (MBNL) proteins are key regulators of alternative splicing (AS), essential for tissue development and cellular differentiation. Among the three paralogs, MBNL1, MBNL2, and MBNL3, MBNL1 plays a predominant role in skeletal muscle function. In myotonic dystrophy type 1 (DM1), toxic RNA transcripts containing expanded CUG repeats sequester MBNL proteins into nuclear foci, leading to their functional depletion. This results in widespread splicing defects, impaired cellular processes, and highlights the urgent need for therapeutic strategies, such as enhancing MBNL1 production, to mitigate disease progression.

While MBNL1 regulation has been extensively studied at the transcriptional and splicing levels, its post-transcriptional control remains poorly understood. Notably, the 5' untranslated region (5'UTR) of the MBNL1 transcript spans approximately 2 kb and contains multiple upstream open reading frames (uORFs), a common but underexplored mechanism of translational regulation. uORFs can modulate translation of the main coding sequence in response to developmental or environmental cues.

Our research aims to identify functional uORFs within the MBNL1 5'UTR that fine-tune MBNL1 protein expression. Using publicly available global ribosome profiling (Ribo-seq) data, we mapped translation initiation sites and ribosome occupancy, revealing several candidate uORFs with potential regulatory roles.

To assess their biological function, we developed a series of plasmid-based reporter constructs incorporating different promoter contexts (CMV and endogenous MBNL1) and luciferase assay systems. These constructs were tested across multiple cell lines to evaluate tissue-specific effects on translation. Additionally, we mutated start and stop codons of individual uORFs to determine their impact on translational efficiency by measuring changes in reporter activity.

Our findings suggest that specific uORFs within the MBNL1 5'UTR act as cis-regulatory elements, modulating translation in a context-dependent manner. This layer of regulation may contribute to the tissue-specific expression of MBNL1 and its dysregulation in DM1, offering a potential therapeutic target.

P67

Investigating the Impact of ADAR1 on HCV Replication

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Hepatitis C virus (HCV), a Flaviviridae family member with a +RNA genome, remains a global health challenge despite the effectiveness of new direct-acting antivirals (DAAs). A preventive vaccine is still lacking. This report explores the interplay between HCV and adenosine deaminase acting on double-stranded RNA 1 (ADAR1), an innate immunity enzyme. ADAR1 catalyzes adenosine-to-inosine conversion, influencing dsRNA stability and the nucleotide sequence. To investigate ADAR1's impact on HCV replication, we established an ADAR1 knockout cell line from Huh7.5 hepatocellular carcinoma cells. We will present the initial findings from HCV replication experiments in this KO cell line.

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P68

How Redox Stress Shapes the Behavior of Wobble 2-Selenouridines and 2-Thiouridines

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Post-transcriptional modifications at the wobble position (U34) of tRNA play a crucial role in maintaining the fidelity and efficiency of mRNA decoding during protein biosynthesis. The hypermodified nucleoside 5-methylaminomethyl-2-selenouridine (mnm5Se5U) is a rare selenium-containing modification present in bacterial tRNAs. While the functional effects of selenium incorporation into RNA are not yet fully understood, 2-selenouridines are thought to provide protective benefits during oxidative stress by modulating redox reactivity and maintaining fidelity of translation.

In this study, we focus on the reactivity of mnm5Se2U under both oxidative and reducing stress conditions that simulate physiologically relevant environments. We have already demonstrated the susceptibility of 2-selenouracil (Se2Ura) and 2-selenouridine (Se2U) to redox transformations. [1,2] Building on this, we now provide a comprehensive analysis of mnm5Se2U oxidation products using high-resolution liquid chromatography–mass spectrometry (LC-MS). Our results show specific oxidative degradation pathway of selenium -containing nucleoside.

Besides, using cyclic voltammetry we performed electrochemical studies to compare the redox profiles of Se2U and S2U. The voltammograms show different redox potentials, suggesting that selenium presence alters the electron transfer properties of the nucleoside in comparison to behavior of thio-analog. These differences may be crucial for redox sensing or protective functions within the cell, especially during oxidative stress. This work provides new insights into the chemical behavior of chalcogen-containing RNA modifications and lays the foundation for understanding their functional significance in stress responses and translational control.

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P69

Expression and purification of Streptococcus pneumoniae proteins KhpA and KhpB KH_R3H and analysis of their interactions with sRNA CcnD.

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KhpA and KhpB are RNA-binding proteins conserved in many Gram-positive bacteria, including Streptococcus pneumoniae, where they influence cell division and bind overlapping sets of RNA targets, suggesting a potential function as a heterodimer. Although they have been proposed to act as RNA chaperones—similar to Hfq and FinO-domain proteins in Gram-negative bacteria—the mechanisms by which KhpA and KhpB recognize RNA remain poorly understood. Unlike other KH domain proteins such as NusA or Era, which display defined nucleotide preferences, no specific structural or sequence motifs have yet been identified for KhpA or KhpB, leaving their RNA-binding specificity largely unexplored.

To begin addressing these questions, we focused on the recombinant production and characterization of KhpA and a truncated variant of KhpB from S. pneumoniae. For KhpB, a construct containing only the KH and R3H domains was designed to exclude an unstructured linker region present in the full-length protein, which may increase susceptibility to aggregation. Initial attempts using the pET-15b vector were problematic due to leaky expression prior to IPTG induction and apparent toxicity. To overcome this, we adopted the tightly regulated pBAD system, enabling controlled expression upon arabinose induction and significantly improving protein yield and stability.

To monitor the binding of both proteins to regulatory RNA CcnD we used electrophoretic mobility shift assays (EMSA). Binding assays revealed strong interaction with CcnD RNA only when both KhpA and KhpB_KH-R3H were present. KhpB_KH-R3H alone showed weaker binding, while KhpA alone did not bind at all. Interestingly, we observed the same mobility shift for RNA-protein complexes in reactions that contained only KhpB_KH-R3H and in reactions that contained both KhpA and KhpB_KH-R3H suggesting that KhpB_KH-R3H may bind CcnD RNA as a homodimer.

These results provide new insight into the RNA-binding behavior of KhpA and KhpB, offering a foundation for further structural and functional studies.

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P70

ATP hydrolysis activity of human ribonuclease Dicer

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Vertebrates have one Dicer ortholog that generates both microRNAs (miRNAs) and small interfering RNAs, in contrast to the multiple Dicer-like proteins found in flies and plants. Our studies focus on the functions of the human Dicer (hDicer) helicase domain. The helicase domain of hDicer is known to recognize pre-miRNA substrates through interactions with their apical loop regions. Besides interacting with canonical substrates, the hDicer helicase domain has also been suggested to bind many different cellular RNAs; however, a comprehensive study of the biochemical activities and substrate specificity of the hDicer helicase domain towards different nucleic acids has yet to be undertaken. We conduct such an analysis and reveal that full-length hDicer, through its helicase domain, hydrolyzes ATP. To the best of our knowledge, this is the first time this activity has been reported for vertebrate Dicers. *In vitro*, the ATPase activity of hDicer can only be observed under low-turnover conditions. We also show that the hDicer helicase domain binds single- but not double-stranded nucleic acids, which is in contrast to the helicase domains of invertebrate and plant Dicers interacting with RNA duplexes. Moreover, the hDicer helicase domain might influence the structure of the bound RNA. Our findings open new avenues for future studies aimed at defining the cellular activities of hDicer that may be associated with these newly described biochemical properties.

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P71

Non-canonical translation of splicing factors in inflammation

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Inflammation is the body's response to harmful and foreign agents, playing a key role in initiating the healing process. However, when it persists over an extended period, it becomes chronic, leading to the pathological states characterized by the infiltration of inflammatory cells into affected tissues. Here, I uncover molecular drivers of inflammation and define the principles of translational regulation within the cellular machinery of the spliceosome. By doing so, I hope to lay the foundation for informed therapeutic approaches to combat inflammation-related diseases, which are becoming prevalent in modern societies.

We recently discovered that translation of spliceosome constituents – splicing factors (SFs), is controlled by methylation of encoding mRNAs within the regulatory portion of 5'-untranslated region. Dynamic changes of methylation offset translation of regulatory SFs to dictate rates of cancer progression. Our preliminary data have unexpectedly identified Interferon-induced proteins with tetratricopeptide repeats 2 and 3 (IFIT2/3) as suppressors of SF translation. This repression depends on methylation status. While IFIT proteins are believed to act solely in limiting the viral infection, recent studies implicated them also in cancer progression and metastasis. However, molecular underpinnings of these observations for inflammation are not understood.

I hypothesize that IFIT2/3 are repurposed during inflammation to suppress the translation of splicing factors, thereby driving alternative splicing. This may enhance innate and adaptive immune response, e.g., by upregulating splicing-derived pro-inflammatory particles including double-stranded RNA and specific immunogenic neo-peptides absent in normal tissues.

Given that alternative splicing is tightly regulated and its dysregulation is linked to various diseases, this work has the potential to significantly enhance our understanding of the molecular drivers of inflammation and therefore offer informed approaches for treating a wide range of human pathologies.

P72

DSIF factor Spt5 coordinates transcription, maturation and exoribonucleolysis of RNA polymerase II transcripts

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The termination of RNA polymerase II (Pol II) transcription is crucial for polymerase recycling and prevents interference with the expression of neighbouring genes. Pol II transcription termination involves the exoribonucleolytic degradation of nascent RNA by the 5'-3' exonuclease Xrn2. Xrn2 utilizes the unprotected 5'-monophosphate group of the nascent RNA, generated by endonucleolytic cleavage at the poly(A) site, as an entry point. However, the molecular mechanisms of this process, including whether Xrn2 is in contact with the Pol II elongation complex to mediate its dissociation from DNA, are not well-defined. In this study, we demonstrate that Xrn2 interacts with both Pol II and Spt5, a conserved transcription factor that controls Pol II processivity and pausing. We provide evidence that Xrn2 activity is stimulated by Spt5 in vitro, and Spt5-depleted cells exhibit defective transcription termination. Our results support a model in which Xrn2 first forms a stable complex with elongating Pol II to acquire its full activity in degrading nascent RNA. Additionally, Spt5 promotes premature termination, attenuating the expression of non-coding transcripts. In contrast, the lack of Spt5 results in Pol II retention at the promoters of protein-coding genes. Pol II molecules that transcribe without Spt5 exhibit reduced elongation rates and defective pre-mRNA processing. We propose that Spt5 is a central node that controls the production of functional mRNA by directly stimulating the activity of RNA enzymes including Xrn2. This ancient transcription factor prevents the entry of improperly configured Pol II complexes into elongation.

P73

A biochemical view of pre-mRNA 3’-end selection

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The nascent 3'-end mRNA maturation requires endonucleolytic cleavage and polyadenylation, processes called cleavage and polyadenylation (CPA). Reactions are carried out by cleavage and polyadenylation specificity factor (CPSF), cleavage factors I and II (CF I and II), cleavage stimulation factor (CSTF), poly(A) polymerase, and at least in vitro, RBBP6. Majority of the human genes harbor at least two polyadenylation sites that are selected during alternative polyadenylation (APA) events. Depending on the localization APA can result in formation of different mRNA isoforms with various coding potentials, altered length of 3’UTRs and stability. Thus, APA contributes to gene expression variability.

We successfully established reconstituted in vitro cleavage assay in our laboratory. We benefit from it and conduct experiments that are helpful for elucidating the mechanism of pre-mRNA 3’-ends selection. Moreover, we perform pre-mRNA cleavage in HeLa nuclear extract testing various conditions. Altogether, the data will allow us for getting insight how mRNA 3’-ends are formed.

P74

The influence of ionizing radiation on miRNA levels in melanoma cells

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MicroRNAs (miRNAs) are small, non-coding RNA molecules that play an essential role in regulating gene expression at the post-transcriptional level. Depending on the cellular context and tissue type, miRNAs may act as oncogenes or tumor suppressors. Ionizing radiation (IR) is used in radiotherapy to damage DNA of cancer cells and miRNAs contribute to the radiosensitivity of cancer cells.

In the present study, we aim to identify miRNAs with altered expression levels in response to IR in Me45 melanoma cells. Me45 cells were irradiated with 4 Gy, leading to cell cycle arrest at the G2/M phase after 8 hours and an increase in apoptotic cells after 24 hours, as compared to non-irradiated controls. Small RNA sequencing was performed to determine the miRNA expression profile in irradiated and control Me45 cells at 4h and 12h post IR. miR-1260 was shown to be consistently upregulated, whereas miR-933 and a novel miRNA: chrM:11954-11969 were consistently downregulated upon IR. Notably, miR-146a was identified as the most abundantly expressed miRNA in both control and irradiated Me45 cells. To further explore its role, miR-146a was inhibited using lentiviral vectors with the miR-Zip-146a sequence or negative control. These vectors also expressed GFP, enabling tracking of transduced cells by flow cytometry in a GFP competition assay. We demonstrated that suppression of miR-146a led to a progressive decrease in the population of GFP-positive Me45 cells compared to wild-type cells in co-culture, indicating that miR-146a supports cell survival of Me45 cells.

In summary, three miRNAs—miR-1260, miR-933, and novel miRNA chrM:11954-11969—were found to be differentially expressed after irradiation of Me45 cells. Additionally, miR-146a silencing reduced cell survival. Ongoing work is focused on elucidating the specific functions of these miRNAs and the involvement of miR-146a in radiosensitivity of melanoma cells.

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P75

Interactome of Proteins Encoded by Cytoplasmic Linear Plasmids of the Yeast *Kluyveromyces lactis*

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Yeast linear dsDNA plasmids are found in certain yeast species, including *Kluyveromyces lactis, Debaryomyces robertsiae, and Pichia acaciae*. Due to their structural and functional similarity mainly to poxviruses, these plasmids are often referred to as virus-like elements (VLEs). Common VLE characteristics include cytoplasmic localization, compact genome organization, the presence of covalently attached terminal proteins, and independence from the host cell's replication and transcription machinery. The first discovered VLEs were the pGKL1 and pGKL2 plasmids from *Kluyveromyces lactis* (Gunge et al., 1981), which nowadays serve as a model system for yeast linear plasmids.

Only limited information is known about the interactions of proteins encoded by pGKL plasmids. We recently demonstrated the interaction between the proteins forming a putative pGKL-specific transcriptional complex, namely RNA polymerase, capping enzyme, and helicase (Sýkora et al., 2018). To identify further potential protein-protein interactions of pGKL plasmid-encoded proteins, we have developed a system to detect and verify interactions between selected proteins in yeast cells, focusing on proteins encoded by the pGKL plasmids. The core of this system is based on the use of optimized genes and the yeast two-hybrid system method. Subsequent steps include immunoprecipitation procedures followed by analysis using mass spectrometry. For these purposes, a wide range

of pGKL proteins were tagged *in vivo* with various tags. These tagged proteins are intended for use in immunoprecipitation, purification, and localization within the host cell.

In summary, we were able to confirm the previously detected interactions using a modified yeast two-hybrid system, as well as identify new interactions between pGKL-encoded proteins.

Acknowledgement

The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU and Project OP JAC CZ.02.01.01/00/22_008/0004575 RNA for therapy.

P76

Targeting HuR in the Post-Transcriptional Regulation of Alpha-Synuclein: New Strategies for Parkinson's Disease

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HuR (ELAVL1) is a highly conserved RNA-binding protein that plays a central role in post-transcriptional gene regulation by binding to AU-rich elements (AREs) within the 3'-untranslated regions (3'-UTRs) of target mRNAs. Structurally, HuR contains three RNA Recognition Motifs (RRMs) responsible for RNA binding, and an intrinsically disordered region (IDR) that harbors the HuR Nucleocytoplasmic Shuttling (HNS) sequence, critical for its movement between the nucleus and cytoplasm. Under native conditions, HuR is primarily localized in the nucleus, with a fraction residing in the cytoplasm. However, HuR can translocate to the cytoplasm in response to cell cycle progression, inflammatory signals, or DNA damage. Notably, HuR's cytoplasmic shuttling has been implicated in cancer progression and in the immune response to viral infections. Despite these findings, the role of HuR shuttling has not been investigated in the context of Parkinson's Disease, representing a potential regulatory mechanism that remains unexplored in neurodegeneration.

Recent published data suggest that HuR may contribute to Parkinson's pathology

by interfering with the maturation of miR-7, a microRNA known to downregulate alpha-synuclein (SNCA), and by directly stabilizing SNCA mRNA through binding to AU-rich elements in its 3'-UTR. These dual actions of HuR promote increased alpha-synuclein levels, a key pathogenic feature of Parkinson's Disease.

Considering these observations, our research aims to develop pharmacological strategies targeting HuR. Two complementary approaches are pursued: (1) screening and characterization of small molecules that disrupt HuR's RNA-binding activity, and (2) exploration of compounds designed to interfere with the HNS sequence, aiming to impair HuR's ability to shuttle between the nucleus and cytoplasm. By targeting both the RNA-binding and trafficking properties of HuR, this work seeks to diminish its pathogenic influence on alpha-synuclein regulation and to establish new therapeutic directions for Parkinson's Disease.

Our ongoing studies contribute to a broader understanding of RNA-binding protein function in neurodegeneration and highlight HuR as a promising post-transcriptional target for intervention.

P77

UpRoi1: a small non-coding RNA of phage origin with dual regulatory roles in phage and host development

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A novel small RNA (sRNA) termed UpRoi1 was discovered after prophage induction in the Shiga toxin-converting phage Φ24B. This RNA, encoded within the antirepressor region of the phage genome, is remarkably short, containing only 30 nucleotides in length, resampling eukaryotic microRNAs size. Such a small size is unusual among bacterial sRNAs. Bioinformatic predictions revealed that UpRoi1 can potentially interact with multiple targets across both the phage and bacterial genomes, indicating a multifaceted regulatory function. RNA-seq analyses demonstrated that UpRoi1 significantly influences the host's gene expression, especially affecting pathways related to bacterial motility. RNA binding studies confirmed that UpRoi1 can directly interact with the 5' untranslated region (UTR) of the phage antirepressor Roi mRNA, as well as with mRNA encoding the bacterial flagellar protein FlgL. These interactions appear to modulate both phage and host processes, promoting the transition of the phage into the lytic phase. The RNA-binding protein ProQ was found to facilitate the interaction between UpRoi1 and its target RNAs. Taken together, these findings highlight the idea that microRNA-like regulators exist in Shiga toxin-converting phages and play an important role in controlling phage development in *Escherichia coli* cells.

P78

The role of RNA uridylation in transcriptome regulation during quiescence

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Uridylation, the non-templated addition of uridine residues to RNA 3' ends, is a key post-transcriptional modification that regulates mRNA stability in proliferating cells. However, its role in non-dividing states remains poorly understood. Here, we investigate the function of the uridylyltransferase Cid1 during cellular quiescence (G_(0))—a reversible state of metabolic dormancy—using the fission yeast *Schizosaccharomyces pombe* as a model. Quiescence underlies fundamental processes in multicellular organisms, including stem cell maintenance and tissue regeneration. Maintaining transcriptome homeostasis in this state is critical for long-term survival and the ability to resume proliferation.

To assess the role of Cid1 in quiescence, we induced G_(0) via nitrogen starvation and analyzed phenotypic and molecular consequences of *cid1* deletion. The Δ*cid1* mutant displayed reduced viability during prolonged G_(0) and delayed re-entry into the cell cycle following nitrogen replenishment. These defects mirrored those observed in mutants lacking key components of the 5'–3' mRNA decay pathway (Δ*xrn1*, Δ*lsm1*, Δ*edc1*), suggesting a functional interplay between Cid1-mediated uridylation and RNA degradation machinery in quiescence.

To gain mechanistic insight, we applied genome-wide RNA 3' end profiling (gw3'RACE), which revealed progressive poly(A) tail shortening and accumulation of oligouridylated transcripts in wild-type cells entering and residing in G_(0). This molecular signature resembled that of RNA decay-deficient strains, further implicating uridylation in RNA turnover during non-proliferative states. RNA-seq analyses showed marked transcriptional differences between wild-type and Δ*cid1* cells upon G_(0) entry: transcripts related to translation and RNA processing were upregulated in Δ*cid1*, while genes involved in amino acid biosynthesis, non-coding RNA processing, and the Lsm complex were downregulated. These imbalances gradually diminished over time, suggesting a delayed and incomplete transcriptome adjustment in the absence of Cid1.

Our findings reveal that Cid1-driven uridylation contributes to RNA decay and transcriptome remodeling in quiescence, promoting survival and timely cell cycle re-entry. This study expands the understanding of uridylation as a dynamic regulatory mechanism in cellular states beyond proliferation.

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P79

Dysregulation of the miR-181b/CYLD Axis in the Development of Ulcerative Colitis

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Introduction: Ulcerative colitis (UC) is a chronic inflammatory disorder of the colon, characterized by inflammation confined to the mucosal and submucosal layers. Among individuals with primary sclerosing cholangitis (PSC), approximately 70% are also diagnosed with UC. In UC alone, inflammation typically originates in the sigmoid colon, whereas patients with PSC-UC more commonly exhibit involvement of the ascending colon. MicroRNAs (miRNAs, small non-coding RNAs) play key regulatory roles in immune responses and inflammation, and are increasingly recognized as contributors to tumorigenesis. The upregulation of miR-181b inhibits the expression of cylindromatosis lysine 63 deubiquitinase (CYLD) and enhances NF-κB signaling, thereby amplifying inflammatory responses implicated in colorectal carcinogenesis.

Aim: This study aimed to investigate the expression of miR-181b and CYLD mRNA in ascending and sigmoid colon.

Methods: Colon biopsies were analysed in UC, PSC-UC patients and healthy controls (n = 10 per group). The level of CYLD protein was measured in the human normal epithelial colon cell line NCM460D after the experimentally induced overexpression of miR-181b via mimic transfection. The expression of miR-181b and CYLD mRNA were quantified by real-time PCR and CYLD protein levels were assessed by western blot analysis.

Results: In UC, the expression of miR-181b was elevated in the sigmoid colon (2.3-fold, $p = 0.02$ vs. control), while the expression of CYLD mRNA was increased in the ascending colon (1.3-fold, $p = 0.03$ vs. control). A significant negative correlation was observed between the expression of miR-181b and CYLD mRNA in the sigmoid colon of UC patients ($r = -0.6$, $p = 0.01$). No significant expression of the miR-181b/CYLD pathway components was detected in PSC-UC samples. In vitro functional studies confirmed that transfection with a miR-181b mimic led to significant downregulation of CYLD protein levels compared to control cells (60% reduction, $p = 0.0006$).

Conclusions: In patients with UC, the miR-181b/CYLD pathway plays a significant role in sustaining chronic inflammation, supporting the existence of distinct molecular phenotypes between UC and PSC-UC.

P80

Rsp5 ubiquitin ligase is implicated in the control of tRNA processing and RNA polymerase III transcription machinery

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Transfer RNA (tRNA) biogenesis in yeast involves the synthesis of the primary transcript by RNA polymerase III (Pol III), followed by processing to remove 5' and 3' ends, further maturation, and export to the cytoplasm. High levels of unprocessed primary tRNA transcripts in mutants of the ubiquitin ligase Rsp5 under restrictive conditions were previously reported in the literature. Here, we explored a role of Rsp5 in tRNA biogenesis. Northern blotting indicated that the high levels of unprocessed primary tRNA transcripts in *rsp5* mutants at elevated temperature were reduced upon the overexpression of *RPR1*, the catalytic RNA subunit of RNase P. Interestingly, *RRP1* by itself was cleaved in *rsp5* mutants. Under the same conditions, the amount of newly synthesized tRNA decreased as determined by in vivo labeling. These observations suggest a role for Rsp5 in control of the catalytic activity of RNAse P and thus, 5’ processing of tRNA precursors. Furthermore, we found that Rsp5 directly interacted with the Tfc3 subunit of the TFIIIC, a general Pol III transcription factor, which is modified by ubiquitination. The inactivation of Rsp5 catalytic activity affected the recruitment of TFIIIC and Pol III to tRNA genes and the interaction between the Pol III factors, TFIIIC and TFIIIC. These findings suggest that Rsp5 ligase is implicated in the control of Pol III machinery in yeast.

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P81

Biological function of elongated at 5' end CENPL-210 transcript and its head-to-head overlap with *DARS2* in human

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Many genes utilize alternative transcription start sites that lead to the elongation of the 5' ends of transcripts. In turn, the head-to-head overlap with the gene on the opposite DNA strand may occur. This overlapping genomic arrangement could influence the expression of genes involved in the overlap due to, e.g., RNA:RNA duplex formation. In cancer, aberrations in the overlapping genes expression can significantly influence cancer cell biology. Despite ongoing research, the biological significance of transcript elongation at the 5' ends and the overlap between two protein-coding genes remains not fully elucidated. In this study, we investigated the role of 5'-end-elongated transcript CENPL-210, which overlaps the transcripts of the *DARS2* gene. Our results indicate that the CENPL-210 and overlapping *DARS2* transcripts form RNA:RNA duplexes in human cells. Moreover, both *CENPL* and *DARS2* have been identified as oncogenes in various cancer types, including breast cancer. In the non-cancerous MCF-12A breast cell line, CENPL-210 has a lower expression level compared to non-overlapping transcripts of the *CENPL* gene and is located exclusively in the nucleus, suggesting its regulatory function. Studies conducted in the breast cancer cell line MC7-7 further support this regulatory role. The expression silencing experiments revealed that the knockdown of CENPL-210 significantly decreased the expression of both*CENPL* and *DARS2* oncogenes. Additionally, silencing the CENPL-210 transcript reduced breast cancer cell proliferation by diminishing the percentage of cells in the G2/M phase of the cell cycle. Summing up, the 5' end-extended transcript of the *CENPL* gene has an important regulatory role, essential for ensuring elevated expression levels of both *DARS2* and *CENPL* genes. These findings also suggest that the CENPL-210 could be a promising therapeutic target in breast cancer treatment.

P82

The in vivo binding of Sib RNAs to Escherichia coli ProQ is sensitive to mutations in the FinO domain

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The ProQ is a global RNA-binding protein from the FinO family that interacts with numerous RNA targets in Escherichia coli. However, it remains unclear whether these interactions occur through a uniform binding mechanism. To investigate this, we introduced five single amino acid substitutions into the FinO domain of ProQ and analyzed RNA binding in vivo using RIP-seq method.

The results revealed that each mutation had a range of effects on the amounts of different RNAs. Focusing on the antitoxin SibA-E RNAs, we observed that these RNAs exist in two distinct length forms. Moreover, the data showed that the R80K substitution most strongly impaired pull-down of all five Sib RNAs, followed by K54A, while K35A had a moderate effect. Interestingly, the R62A and R69A mutations had differential effects on the binding of individual Sib RNAs, with opposing effects on SibA/B/E compared to SibC/D.

To identify the regions of Sib RNAs responsible for differential pull-downs observed with the R62A and R69A mutants, we constructed chimeric SibA and SibC with swapped transcription terminator structures. Northern blot analysis revealed that the effects of R62A and R69A on RNA pull-downs were dependent on the origin of the terminator structure. Additionally, gelshift assays showed that removal of the transcription terminator from SibA and SibC weakened their binding to full-length ProQ and completely abolished interaction with its N-terminal FinO domain. Furthermore, RNA footprinting analysis of the SibA RNA in the presence of either full-length ProQ or its FinO domain indicated that the binding site is localized at the base of the terminator hairpin structure. These findings indicate that the terminator region plays a crucial role in binding Sib RNAs to ProQ and its mutants.

Overall, our data suggest that ProQ-RNA interactions in E. coli are individually fine-tuned, primarily through interactions between the FinO domain and RNA terminator structures. These findings expand our understanding of RNA selectivity by ProQ and highlight how mutations in a conserved RNA-binding domain can differentially affect RNA binding in vivo.

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P83

Role of the TOM complex in the mitochondrial localization of the components of the LSM complex

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The functioning of mitochondria depends on the activity of proteins encoded by both nuclear and mitochondrial genes. The TOM complex is responsible for the transport of specific nuclear encoded proteins across the outer mitochondrial membrane. The mitochondrial targeting signal (MTS), present at the N-terminus of some proteins, is recognized by the TOM70 subunit, enabling their correct subcellular localization. In certain cases, MTS presequences may be located within an N-terminal extension (NTE) generated by non canonical translation, in which translation is initiated upstream of the canonical start codon. This leads to the formation of longer protein isoforms that may contain additional signal sequences. Notably, in yeast *Saccharomyces cerevisiae* some proteins forming the LSM complexes contain such NTEs with predicted MTS, suggesting their potential mitochondrial targeting. LSM protein complexes, nuclear Lsm2-8 and cytoplasmic Lsm1-7, are involved in various aspects of RNA metabolism, namely pre-mRNA splicing and mRNA decay, respectively. To investigate the role of the TOM complex in mitochondrial targeting of LSM components, we checked localization of Lsm1, Lsm5, and Lsm8 proteins in wild-type and *tom7Δ*, *tom20Δ* or *tom70Δ* strains lacking TOM complex subunits, using cell fractionation into mitochondrial and cytosolic fractions followed by Western blot analysis of C-terminally tagged variants. Additionally, in silico modeling of alternative LSM protein isoforms containing NTEs was performed and compared to known MTS sequences. The results suggest that these extensions may serve as targeting signals for mitochondrial import. The presence of selected LSM proteins in mitochondria indicates a broader spectrum of mitochondrial RNA processing and/or decay mechanisms than previously described. Our results will reveal whether import pathways of noncanonical proteoforms into mitochondria involve the TOM complex.

P84

Naphthyridine carbamate dimer ligand induces formation of Z-RNA-like fold of disease-related RNA and serves as a molecular glue for crystal lattice formation

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RNA has emerged as a promising therapeutic target against specific neurological disorders such as spinocerebellar ataxia type 31 (SCA31). The SCA31 is caused by expansion of repeat sequence TGGAA/TGGAA, located in the 3' untranslated region (UTR) of the BEAN1 gene. The mutated gene results in mRNA containing expended sequence motif which contributes to the SCA31 pathogenesis. Recent advancements in understanding the molecular mechanisms underlying SCA31 have opened avenues for the development of small molecule therapeutics targeting the UGGAA/UGGAA repeat sequence. Small molecules offer the potential to modulate the aberrant RNA structure or interfere with the pathogenic RNA-protein interactions associated with SCA31 [1, 2].

The prof. Nakatani research team, have identified promising small molecules using screening assays. They developed naphthyridine derivative molecule, which exhibits robust affinity for G-rich RNA sequences. The naphthyridine carbamate dimer (NCD) binds RNA containing UGGAA/UGGAA motifs and reduce the disease phenotype in Drosophila model of SCA31 [3, 4].

In this study, we present the structural analysis of the NCD ligand bound to RNA containing the UGGAA/UGGAA motif. We characterized two crystal structures of RNA-ligand complexes, along with unliganded RNA structure. In the complex models, we observed two NCD molecules bound to the internal loop formed by the UGGAA motif. Furthermore, in one of the complexes the additional binding site of NCD was found. The ligand was located between symmetry-related RNA molecules and helped to form additional interactions in the crystal lattice. This showed that NCD ligand can be used as molecular glue to facilitate the formation of crystals.

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P85

Targeting segment 8 of Influenza A Virus vRNA with Antisense Oligonucleotides

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RNA viruses pose a serious threat to public health due to their high genetic variability and ability to spread rapidly. The influenza A virus (IAV), one of the most commonly occurring RNA viruses, causes seasonal epidemics and influenza pandemics. The genetic material of IAV consists of 8 segments, in which the presence of conserved structural motifs has been identified. Finding effective therapeutic strategies to combat the influenza A virus (IAV) is crucial in growing resistance to available antiviral drugs. This study evaluated the efficacy of antisense oligonucleotides targeting conserved motifs within vRNA segment 8 in inhibiting IAV replication. The antisense oligonucleotides used were conjugated to compounds such as cholesterol, GalNac, and palmitate to increase intracellular delivery efficiency. This modification allowed their autonomous uptake into cells. In addition, the antisense oligonucleotides were labeled with cyanine (Cy3), enabling analysis by confocal microscopy. The results showed effective entry of the antisense oligonucleotides into cells, confirming their ability to be independently internalized. Based on the obtained images, it is possible to determine the location of the applied antisense oligonucleotides within the cells. The Immunofluorescence Focus Formation Assay (IFFA) results showed significant inhibition of IAV replication. In addition, cytotoxicity tests showed no toxic effects of the analyzed antisense oligonucleotides on cells. The study confirms the potential of the used antisense oligonucleotides as effective inhibitors of influenza virus replication, which may represent a promising therapeutic strategy for treating and preventing influenza infections.

P86

Shedding new light on lncRNA functions in vertebrates

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Accurate and complete gene annotations are essential for understanding how genome sequences encode biological functions. For two decades, the GENCODE consortium has provided reference annotations for human and mouse genomes, serving as a cornerstone for biomedical and genomics research. However, long non-coding RNAs (lncRNAs) remain under-annotated and dispersed across multiple, uncoordinated catalogs, hindering progress in functional genomics.

To address this challenge, GENCODE has undertaken the most comprehensive lncRNA annotation effort to date. This initiative leverages manual annotation of full-length targeted long-read sequencing data from matched embryonic and adult tissues, capturing orthologous regions in human and mouse. This rigorous approach significantly expands the GENCODE catalog with novel lncRNA genes and transcripts.

Our annotation effort has identified 17,931 novel human genes (140,268 novel transcripts) and 22,784 novel mouse genes (136,169 novel transcripts), representing a 2-fold and 6-fold increase in transcripts, respectively—the most substantial expansion since the human genome was first sequenced. These novel lncRNAs exhibit evolutionary constraints, well-defined promoter regions, and connections to phenotype-associated genetic variants. They also enhance the functional interpretability of the human genome by explaining millions of previously unmapped “orphan” omics signals, including transcription start sites, chromatin modifications, and transcription factor binding sites. Importantly, our targeted annotation strategy dramatically improves human-mouse ortholog assignment, tripling the number of human disease-associated lncRNAs with mouse counterparts.

The expanded and refined GENCODE lncRNA annotations mark a significant advancement in genome research, bridging the gap between sequence annotation and functional interpretation. These annotations provide a critical resource for exploring lncRNA function, evolutionary conservation, and their roles in human disease. This work underscores the necessity of continuous and coordinated annotation efforts to fully decipher the complexity of the human and mouse genomes.

P87

Cryo-EM SPA of non-coding RNAs.

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Our understanding of the diverse roles of RNAs is growing exponentially. Ribonucleic acids (RNAs) play numerous, distinct and vital roles in biological systems. RNAs, like proteins, have the ability to fold into higher order structures. Nonetheless, structures of proteins represent the vast majority of PDB depositions and less than 1% of available entries describe RNA-only structures. RNA structure determination is complex due to multiple factors and complicated by the inherent ability of RNA domains to adapt multiple conformations. RNA domains in both catalytic and non-catalytic RNAs fold into largely unknown three-dimensional structures. Although sequence information and folding predictions for structured RNAs is widely available, efficient structural determination lags behind, hindering their holistic molecular characterization. In this talk, I will discuss the cryoEM-SPA of aptamer domain of the GlmS-ribozyme and Hammerhead-10 ribozyme. Both the ribozyme structures play an important role in physiological context and have potential biotechnological applications. In addition, I will discuss the neuron specific tRNAArgUCU a single point mutation at position 50 in the sequence (C50U) was reported to play a role in neurodegeneration in mice. It was shown by our collaborators in an earlier study that the single point mutant variant stabilizes alternative conformations that effect the processing by RNase P and thus contributes to defects in neuronal homeostasis. I will present the conformational ensembles that we obtained, at a limited resolution, for such a small molecule (23kDa) using cryoEM-SPA.

P88

Benchmarking the effect of divalent and monovalent metal ions on RNA structure

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RNA molecules are crucial for a variety of biological functions due to their ability to adopt complex structural folds. Stability and functionality of such complex molecules is closely related to the presence of counterions neutralizing the backbone, both mono- and divalent ions. While the role of the most abundant Mg^{2+} ion in shaping the RNA molecule is well established, recent studies show that other ions may also play significant role in RNA catalysis and folding. While dedicated methods towards detection of ions in complex systems are being developed, recent evidence shows that some of the Mg^{2+} ions may be substituted with K^{+} ions.

In my project I employed both classical and quantum-classical molecular dynamics (MD) simulations to determine the function of various divalent and monovalent ions in selected RNA systems differing in size and function, whose experimental structures have been reported in the presence of divalent and/or monovalent ions. The challenge in the accuracy of the MD simulations lies in the accuracy of force fields used to parameterize the monovalent and divalent ions and their interactions with the phosphate backbone. Currently, multiple parameterizations for both mono- and divalent ions are available, therefore my plan is to assess the performance of the developed parameters and establish a dependable set of parameters for both monovalent and divalent metal ions that can be effectively and reliably utilized to perform MD simulations of RNA systems and aid in metal ion assignment. While RNA molecules have gain importance in various medical and biochemical applications, the outcome of such a project will become beneficial in biochemistry, biotechnology, and medicine, potentially influencing future research and therapeutic strategies.

P89

The effect of selenium nanoparticles and cross-talk of selenium nanoparticles and *Fusarium oxysporum* f. sp. *lupini* on soluble sugar content in *Lupinus luteus* L., expression of genes involved in abscisic acid biosynthesis and cell ultrastructure

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The present research focuses on the role of selenium nanoparticles (SeNPs) at hormetic dose in the regulation of yellow lupine defense responses to the biotic stressor, i.e. hemibiotrophic pathogen *Fusarium oxysporum* f.sp. *lupini* . The experimental protocol used in this study was based on a model system: the germinating seeds pre-treated with SeNPs or non-treated, and inoculated with a *F. oxysporum* f. sp. *lupini* spore suspension or non-inoculated and cultured on the Hoagland medium. The first aim of this study was to investigate changes in soluble sugar concentration in intact embryo axes of germinating seeds and seedling roots. The second aim was to determine expression of genes encoding enzymes of the biosynthesis of abscisic acid (ABA). Real-Time qPCR was applied to analyse the expression of genes encoding enzymes involved in the regulation of biosynthesis of ABA [9-cis-epoxycarotenoid dioxygenase (NCED) and zeaxanthin epoxidase (ZEP)]. Additionally, ultrastructural analyses of root cap cells of yellow lupine (*L. luteus* L. cv. Diament and *L. luteus* L. cv. Mister varieties) in transmission electron microscopy (TEM), the Hitachi HT7700 (Hitachi, Tokyo, Japan) were performed. The obtained research results demonstrated that soluble sugars, especially sucrose, glucose, and fructose are involved in the responses to *F. oxysporum*, and they act as signaling molecules that activate specific or hormone-crosstalk transduction pathways, triggering changes in gene expression.

P90

miRNA-guided RNA based therapeutics target key resistance pathways in Multiple Myeloma.

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Background: Recent advances have improved outcomes for patients with multiple myeloma (MM), but the disease remains incurable. Most patients eventually experience relapse due to minimal residual disease and drug resistance. To address these challenges, we are developing RNA-based therapeutics that modulate dysregulated microRNAs (miRNAs) involved in the pathogenesis of MM. This strategy aims to be cost-effective, scalable, and adaptable for clinical applications.

Approaches and Results: Our approach focuses on three miRNAs that are frequently altered in MM: miR-193b, which is upregulated in over 50% of patient samples, and miR-125 and miR-26, which are downregulated in approximately 45% to 85% of cases, respectively. We selected these miRNAs because our in-silico analyses suggest that modulating them in combination can regulate CDK6, NOTCH1, and CCND1, which play crucial roles in the survival, proliferation, and resistance of MM cells to therapies. We have designed mixed types (RNA/DNA) of oligonucleotides to restore the physiological expression of these miRNAs. In vitro treatment resulted in a rapid downregulation of the target genes at both the transcript and protein levels within 24 hours, inducing approximately 50% cell death in MM cells, even with low transfection efficiency. Mechanistically, suppression of CDK6 and CCND1 disrupts abnormal cell cycle progression, while NOTCH1 inhibition impairs MM cell interaction with the bone marrow stroma and reduces immune evasion, affecting T-cell responses and cytokine signaling.

In conclusion, preclinical studies are ongoing to evaluate the efficacy of these RNA drugs in both in vitro and in vivo models. Our data support a novel, miRNA-guided RNA therapeutic platform that simultaneously targets multiple resistance pathways, with strong potential to enhance treatment durability and outcomes in relapsed or refractory MM.

P91

Dual function of the C regulator: transcription factor and regulatory RNA in restriction endonuclease expression within C protein-linked Type II restriction-modification systems

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Restriction-modification (R-M) systems are highly abundant among bacteria and Archea, playing crucial roles in modulating horizontal gene transfer and protection against invasive DNA, such as bacteriophages. These diverse enzyme systems, including their regulatory mechanism, remain an active area of exploration.

Type II R-M systems consist of genes coding for separate proteins with independent enzymatic activities: a restriction endonuclease (REase) and a protective DNA methyltransferase (MTase). An imbalance between REase and MTase activity can be co lethal to the host, necessitating tight regulatory mechanisms for gene expression. Some R-M systems include an additional gene coding for a transcription factor called a C-protein (C – controlling). C proteins play a vital role in the temporal regulation of R-M gene expression, as well as its function to influence the R-M system's mobility and stable maintenance in the bacterial population.

In the Csp231I R-M system, the C-protein acts primarily as an auto-repressor by binding to a conserved DNA sequence (the C-box) in its promoter region creating a negative feedback loop. Transcription activation or repression depends on cellular C protein concentration, though inhibition dominates across a broad concentration range. Further studies revealed that the level of C transcript is negatively regulated by the antisense RNA driven from reversed promoter PaC located in the complementary strand within the C gene. Inactivation of this PaC promoter leads to drastic increase of C gene expression and a significant reduction of REase mRNA levels resulting in the low relative restriction. Disturbance in R-M systems gene expression through absence of antisense RNA or C gene deletion results unexpectedly in loss of R-M system stability regardless its relative restriction activity.

These observations could provide the new insights into the mechanisms that control gene expression of selfish genetic elements. The presence of a possible multi-layered complexity is not surprising as R-M systems like other toxin-antitoxin modules must be controlled to keep the counter-balancing amounts and timing properties to avoid lethality.

P92

Modulation of the endogenous *MBNL1* expression via RNAa mechanism as an alternative therapeutic approach towards myotonic dystrophy type 1 (DM1).

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In myotonic dystrophy type 1 (DM1), *DMPK (DM1 Protein Kinase)* transcripts carrying expanded CUG-triplet repeats (CUGexp RNA) sequester Muscleblind-like (MBNL) proteins, impairing their function and shifting the alternative splicing (AltS) pattern of hundreds of target RNAs from adult to fetal splice isoforms (DM spliceopathy). Proteins translated from these mis-spliced mRNA isoforms are functionally unsuitable in an adult organism, thus leading to a plethora of pathological hallmarks including myotonia, muscle atrophy and wasting, insulin resistance and cataract, among others. To overcome MBNL proteins insufficiency in DM cell models, we harnessed an evolutionarily conserved endogenous mechanism of RNA activation (RNAa) via small activating RNA (saRNA) targeted to the most active promoter of *MBNL1* gene, the major paralog of the *MBNL* gene family. We identified two lead saRNA duplexes that stimulated *MBNL1* transcription via an on-site mechanism that involves AGO2-mediated loading of the antisense strand onto target sequence, followed by recruitment of RNAPII and auxiliary canonical RNAa pathway components. Our data highlight transcription factors whose binding recruitment via identified saRNAs affects MBNL1 expression. Using a variety of molecular and biochemical approaches we provide detailed insights in to the underlying mechanism, including the role of cryptic promoter associated RNAs as well as the lncRNA *MBNL1-AS1* overlapping *MBNL1* promoter. Most importantly, we show that RNA activation enhances MBNL1 protein content and corrects the alternative splicing defects of multiple MBNL1 target pre-mRNAs in distinct cellular models of DM1, including primary fibroblasts as well as myoblasts. Overall, this is the first report that site-specific augmentation of the endogenous *MBNL1* transcription mitigates disease-associated AltS defects, hence it offers brand new perspectives in DM1 therapeutic options as well as in *MBNL* expression regulation.

P93

G-quadruplexes within the influenza A virus genome interacting with G4-specific ligands – potential antiviral targets?

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Influenza A virus (IAV) causing pandemic outbreaks is a significant research subject. Despite high genome variability, the viral RNA (vRNA) secondary structure retains consistent features across strains, and its role in the viral life cycle has been described. Among the vRNA structures, G-quadruplexes (G4s) have been confirmed to have function during viral replication and thus are investigated as potential drug targets. We searched the IAV genome for potential quadruplex-forming sequences (PQSs), studied their ability to fold into G4s, and their role in the viral life cycle. We identified PQS twelve motifs within the IAV vRNA. Then we determined their propensity to form G4s using spectroscopic methods (UV, 1H NMR, and CD), and native PAGE. Next, we examined via RT stop assay, if selected motifs interact with G4-specific ligands. Studies in cell cultures allowed us to observe the influence of G4-specific ligands on IAV replication. We conducted our research using two models – the IAV minireplicon system and IAV infection. Our results revealed that three PQSs form stable G4s within protein-coding segments and that G4-specific ligands interact with the selected G4s. Moreover, G4-specific ligands inhibit IAV minireplicon replication and have an impact on IAV infection. We concluded that G4s are present within the IAV genome and can be targeted by ligands leading to viral replication inhibition. Our findings suggest that PQS motifs can serve as potential antiviral targets.

P94

Cajal Bodies Modulate Intron Retention in Response to Hypoxic Stress

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Cajal bodies (CBs) are multifunctional nuclear subdomains involved in the metabolism of various types of RNA, including snRNA and snoRNA, and, as recently demonstrated, also mRNA. The aim of this study was to investigate the role of CBs in transcriptome regulation, including alternative splicing, in plants exposed to hypoxic conditions. RNA-seq analyses were performed on total cellular and nuclear RNA isolated from wild-type (WT) and ncb-1 mutant plants. ncb-1 mutants lack CBs due to a mutation in the coilin gene, which encodes coilin—a marker protein of Cajal bodies.

Fold changes (FC) were assessed for two comparisons: normoxia vs. hypoxia, and hypoxia vs. reoxygenation. In addition to shared responses, genotype-specific differences in response to hypoxic stress were observed. Notably, a substantially higher number of genes were differentially expressed in wild-type (1,696) compared to ncb-1 (516), indicating a potential role of Cajal bodies in transcriptional activity under low oxygen conditions. Next the intron retention (IR) index was used to assess changes across three conditions. Hierarchical clustering revealed seven IR clusters in wild-type (WT) and five in ncb-1. Two WT clusters and one in ncb-1 showed low retention in normoxia and reoxygenation and high in hypoxia. Nuclear transcriptome analysis revealed that many of these IR isoforms were retained in the nucleus during hypoxia, where NMD system is non-functional. Significantly more IR isoforms were retained in wt nuclei than ncb1. The high retention under hypoxia indicates an accumulation of intron-containing mRNAs, potentially serving as a reservoir for rapid post-transcriptional splicing upon reoxygenation. The higher number of IR-associated genes in WT may contribute to more efficient recovery from hypoxic stress. This may underlie the greater hypoxia tolerance and more efficient recovery observed in WT compared to ncb-1.

Finally, RNA immunoprecipitation (RIP) results in WT and ncb-1 support the hypothesis that differences in intron retention linked to the role of CBs in snRNP maturation. The reduced level of snRNPs in ncb1 may therefore lead to pre-mRNA splicing disturbances and differences in intron retention.

P95

RNA-Bender: A Novel Molecular Dynamics-Based Approach for Rapid Sequence-Based RNA Model Construction and Refinement.

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We have developed a novel molecular dynamics-based approach, the RNA-Bender program, designed to facilitate the rapid, sequence-based construction of RNA models. Initiating the folding process with RNA-Bender requires detailed information regarding the shape of the RNA molecule, such as that determined by Cryo-EM, and base pairing data obtained from NMR measurements. This method allows for the controlled folding of RNA molecules by manipulating various fragments, such as bases, loops, and hairpins, in a sequence defined by the user. To prevent unwanted energetic effects that could compromise the final RNA structure during manipulation, the structure is cooled in a controlled manner. We have successfully applied this method to resolve RNA structures obtained from Cryo-EM, refining them to angstrom-level resolution. We intend to present successful case studies and discuss the challenges our approach still faces.

P96

Regulation of Pol II transcription by the deNADding enzyme DXO1 and the 5'-3' exonuclease XRN3 in *Arabidopsis*.

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The XRN and DXO protein families comprise evolutionarily conserved enzymes involved in RNA metabolism. XRN 5'-3' exoribonucleases regulate transcription termination, RNA maturation and quality control. The Arabidopsis XRN3 degrades nascent transcripts, contributes to RNA polymerase II (Pol II) termination, and is involved in rRNA processing and RNAi. In turn, the DXO proteins exhibit diverse enzymatic activities that vary depending on the organism and include 5'-3' exoribonuclease, pyrophosphohydrolase and decapping of either canonical m7G or non-canonical caps. They contribute to RNA cap quality control, RNA decay, rRNA processing, and transcription termination. Plant DXO1 is different from its fungal and animal counterparts. An amino acid substitution near the active site of Arabidopsis DXO1 strongly reduces its 5'-3' exoribonuclease activity but preserves the ability to remove the non-canonical NAD+ cap (deNADding). In addition, DXO1 has a plant-specific N-terminal extension crucial for its functions, e.g. interaction with the mRNA cap methyltransferase RNMT1 responsible for the synthesis of the m7G cap.

We have recently demonstrated that DXO1 and XRN3 cooperate functionally, although not through direct protein-protein interaction. Molecular and morphological phenotypes observed in dxo1-2 mutant plants can be partially reversed by knocking down XRN3, and, conversely, the phenotypes of mutants with silenced XRN3 can be alleviated by DXO1 disruption. Both DXO1 and XRN3 are involved in Pol II transcription regulation: DXO1 in the early step (cap methylation), while XRN3 in the final step (termination). Therefore, we focused on studying this process using single and double mutant lines and plants expressing tagged variants of these enzymes. We have shown that DXO1 interacts with the CTD of Pol II and influences its phosphorylation status, which may be related to defects in capping. We propose that DXO1 may recruit RNMT1 to Pol II for cap methylation. In the absence of DXO1, transcription elongation may be impaired, facilitating termination, which could explain the reduced termination defects observed in the dxo1-2 xrn3-8 double mutant compared to the single xrn3-8 line. In turn, altered polymerase activity resulting from XRN3 silencing may provide a wider time window to restore productive cap synthesis, suppressing dxo1-2 phenotypes associated with inefficient mRNA capping.

P97

Suppression of LINE-1 retrotransposition by retrogene encoded proteins

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LINE-1 retrotransposons are mobile genetic elements that multiply themselves by a copy-and-paste mechanism involving RNA intermediates[1]. LINE-1 is the only autonomous human retrotransposon that encodes the molecular machinery responsible for mobilization of: itself, other retrotransposons, and occasionally other mRNAs leading to formation of so called retrogenes, intronless genes derived from spliced and LINE-1-reverse transcribed mRNAs. RBMXL1, RBMXL2, RBMXL3 are retrogenes encoding RNA binding motif proteins. They derive from the source RBMX a conserved X-chromosome encoded gene. All introns in the coding sequence have been processed out compared to the RBMX locus. RBMXL3 is primate-specific[2]. It comprises a large repeat-rich central domain not present in RBMXL1 and RBMXL2 which are found in multiple organisms including in mouse. Curiously, it has been observed that RBMXL2 and RBMXL3 are specifically expressed during spermatogeneis in mouse and/or in human in spermatogonia, spermatocytes and spermatids [Human Protein Atlas]. Genomic depletion of RBMXL2 resulted in male mice sterility[3]. In humans RBMXL3 mutations were linked with nonobstructive azoospermia in patients resulting in spermatogenesis failure[4]. Intrigued by the expression pattern of the retrogenes which corresponds with the LINE-1 transcriptional activation in spermatogenesis we decided to test whether RBMXL paralogs expression would affect LINE-1 retrotransposition in a cell-based assays in 293T and HeLa cells. We cloned the RBMXL paralogs into overexpression plasmids and cotransfected these with LINE-1 retrotransposition reporter plasmids. We observed efficient expression of all of the paralogs which severely suppressed LINE-1 retrotransposition. This was accompanied by moderate reduction of LINE-1 reporter mRNA, though the phenotype was different depending on the reporters' promoter sequence. Also, the effect seemed independent of LINE-1 protein expression as RBMXL3 presence coincided with higher L1-ORF1p levels. The studies set stage for further investigation of a proposed functional involvement of RBMXL retrogenes in LINE-1 regulation. Financing by the National Science Center, grant 2019/33/B/NZ1/02260 to ZW.

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P98

Structural and Functional Characterisation of FASTK Family Proteins in Mitochondrial RNA Metabolism

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The FASTK (Fas-activated serine/threonine kinase domain containing protein) family of proteins plays a crucial role in the regulation of mitochondrial RNA metabolism, a process essential for mitochondrial gene expression and overall cellular energy homeostasis. Despite their recognized involvement in mitochondrial RNA processing, maturation, and translation, the molecular mechanisms by which FASTK proteins exert their functions remain poorly understood. Dysfunction in these proteins has been implicated in a range of human diseases, including mitochondrial encephalomyopathies and neurodegenerative disorders, underscoring their significance in human health. This project aims to elucidate the structural and functional properties of FASTK family members.

Using an integrative approach that combines cryo-electron microscopy (cryo-EM), complementary biochemical, biophysical and cell-based assays, this research will investigate the domain architecture, RNA-binding specificity, interaction networks of FASTKD proteins and their involvement in mitochondrial molecular mechanisms. High-resolution structural data will be correlated with functional assays, including in vitro RNA processing and cell model studies. In parallel, mass spectrometry and cross-linking analyses will be employed to identify protein-protein and protein-RNA partners. For example, I aim to characterise the mitochondrial isoform of FASTK. To overcome solubility issues commonly encountered with recombinant FASTK expression, I successfully engineered a construct incorporating an N-terminal tag, which significantly enhances protein solubility and stability in vitro. This advancement has enabled downstream applications including preliminary structural studies.

The project will not only provide detailed mechanistic insights into the roles of FASTK proteins in mitochondrial RNA metabolism, but also shed light on how their dysfunction contributes to human mitochondrial diseases. The outcomes will broaden our understanding of mitochondrial gene regulation at the molecular level and have the potential to inform therapeutic strategies targeting mitochondrial gene expression disorders.

P99

The role of mutant polyglycine protein in the pathogenesis of syndromes associated with expansion of CGG repeats in *FMR1* gene

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Fragile X chromosome-associated tremor and ataxia syndrome (FXTAS) is a neurodegenerative genetic disorder caused by an expansion of CGG trinucleotide repeats (CGGexp) involving 55-200 CGG repeats in the 5'UTR region of the *FMR1* gene encoding the FMRP protein, located on the X chromosome. Symptoms develop gradually in the presence of intranuclear protein aggregates in neurons and astrocytes, accompanied by an increase in *FMR1* mRNA levels. Currently, little is known about the exact molecular basis of FXTAS, although several processes have been proposed.

So far, suggested mechanisms of FXTAS pathogenesis include: [1] sequestration by toxic *FMR1* mRNAs from CGG^{exp} of RNA-binding proteins (RBPs) crucial for miRNA maturation and alternative splicing, and [2] biosynthesis of aggregation-prone polyglycine (FMRpolyG) due to non-canonical repeat-associated non-AUG codon-initiated translation (RAN translation).

The project aims to investigate the role of mutant FMRpolyG in FXTAS pathogenesis by deriving stable *in vitro* and *in vivo* models (*Danio rerio*). It seeks to clarify how FMRpolyG aggregates contribute to the disease and to distinguish the effects of toxic protein and RNA from the mutated *FMR1* gene. Understanding these mechanisms is crucial for developing future therapies. The resulting models may serve as tools to test treatments targeting toxic *FMR1* products or reducing protein aggregation.

P100

Characterization of the role of Methyltransferase METTL16 in HIV-1 replication

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Despite antiretroviral therapy (ART), full eradication of the HIV-1 virus is still a challenge due to the persistence of latent viral reservoir. Understanding molecular mechanisms of maintaining and reversing latency is crucial for developing virus eradication strategies. Recent studies highlighted the relevance of N^+-methyladenosine (m^6A) RNA modification pathway in post-transcriptional HIV-1 gene expression. M^6A is the most prevalent, reversible mRNA modification in mammalian cells. The methyl group is added to adenosine by methyltransferase METTL3/METTL14/WTAP "writers" complex and is removed by demethylases "erasers" ALKBH5 or FTO. Modification is recognized by m^6A binding proteins, that decide the fate of the methylated mRNA. It was previously shown that m^6A pathway positively regulates HIV-1 gene expression.

One more "writer" – methyltransferase METTL16 has also been identified, however its role in HIV-1 replication remains unknown. Here, we aim to examine the effect of METTL16 on HIV-1 gene expression and its role in latent viral reactivation. Firstly, using CRISPR/Cas9 approach, we showed that METTL16 negatively regulates HIV-1 gene expression, and its depletion decreases global m^6A levels on mRNA. Next, we showed that METTL16 impacts the stability of viral transcripts using Actinomycin D approach. We also confirmed the binding of HIV-1 RNA to METTL16 using RNA immunoprecipitation and immuno-HIV-1 RNA FISH. Next, to understand the role of METTL16 in latent HIV-1 reactivation, we depleted METTL16 in lymphoid and monocytic in vitro models of HIV-1 latency and we observed potentiated viral reactivation. We also showed that METTL16 inhibition by a selective compound increased viral reactivation in both latency models and ex vivo cultures of $CD8^+$-depleted PBMCs from people living with HIV, mimicking the METTL16 depletion.

Our findings reveal METTL16 as a previously unrecognized negative regulator of latent HIV-1 reactivation and gene expression and suggest METTL16 as a potential therapeutic target for virus eradication.

P101

Aneuploidy-Induced Expression Changes in Trisomy 21 Pluripotent Stem Cells

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Down syndrome or Trisomy 21 (T21) is a genetic disorder caused by an extra copy of chromosome 21 (chr21), resulting in intellectual disability and an increased risk of diseases such as Alzheimer's and leukemia. Although steady-state gene expression studies have demonstrated widespread misexpression of thousands of non-chr21 genes, the impact on core transcription dynamics remains unclear. Previous research reports elevated total RNA levels in T21 cells, yet the underlying mechanism driving this increase is unknown.

Here we hypothesised that increased nascent RNA transcription drives the overall elevation in total RNA levels observed in T21 cells. To test this hypothesis, we set out to compare the transcriptional activity between euploid and trisomic undifferentiated induced pluripotent stem cells (iPSCs) as well as during their differentiation to neural lineages.

To enable these comparisons during differentiation, we first successfully established a robust neural progenitor cell (NPC) differentiation protocol, validated by OCT-4 and Nestin immunofluorescence staining at specific developmental stages. Then, to quantify nascent RNA production at single-cell resolution, we optimized an Click-iT metabolic labelling combined with fluorescence microscopy and automated image analysis.

Preliminary data demonstrated significantly higher nascent RNA levels in trisomic cell lines compared to diploid controls, supporting the hypothesis that elevated total RNA levels in T21 cells arise from increased nascent RNA production.

These initial results provide evidence that increased chr21 dosage drives higher nascent transcription in T21 cells, indicating that hypertranscription may be an inherent consequence of T21. Future investigations will further assess stage-specific transcriptional dynamics during differentiation from iPSCs to NPCs, enhancing our understanding of the developmental impacts of aneuploidy.

P102

WHIRLY1 gene is required for proper gene response to Pi starvation in barley.

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WHIRLY1 (*WHY1*) gene encodes protein which can bind to DNA/RNA and can be directed to chloroplast and nucleus. In chloroplast WHIRLY1 is involved in genome stability maintenance. Phosphorus (P) is a macronutrient required for nucleic acids synthesis, phospholipid membrane formation, ATP synthesis and protein phosphorylation. Phosphorus is acquired by plants as inorganic phosphate (Pi) ions. We found that *WHIRLY1* gene is required for correct gene response to Pi starvation in barley. Using different barley *WHIRLY1* lines (RNAi (KD), CRISPR/Cas9 (KO) and *WHY1* overexpressing line (OE)) we analyzed Pi-related genes expression in response to different Pi regime. Unexpectedly, we found that genes induced under Pi starvation in wild type (WT) plants like *MIR399*, *Induced by Phosphate Starvation 1* (*IPS1*) do not respond to Pi starvation in *WHY1* KO and KD barley lines. The level of unresponsiveness is correlated with the decreased level of *WHIRLY1* gene expression. In *whirly1* KO mutant, the induction is almost completely blocked. The level of mature miR399 in KO mutant is almost 30 times lower compared to the WT plant when grown without Pi in hydroponic conditions. In *whirly1* KO mutant the other canonically Pi starvation induced *MIR827* gene exhibited constant up-regulation of its primary transcript level regardless of the Pi regime. Interestingly the level of mature miR827 is almost not induced compared to WT plants when growing in hydroponic culture without Pi. Consequently, *SPX-MFS1* mRNA level, which is targeted by miR827, is also almost unchanged in KO and KD plants. In the case of *WHIRLY1* overexpression plants we observed similar gene response to Pi starvation like in WT plants. Unlike WT plants, *why1* KO plants show dramatical decrease in chlorophyl content when grown in control and Pi deficient conditions. Regardless of Pi regime, total plant area in the case of *why1* KO plants is also dramatically reduced compared to WT plants. Our results point to extremely interesting observation of the *WHY1* role in posttranscriptional regulation of Pi starvation responsive microRNAs. Further studies are planned to elucidate the molecular mechanism of *WHY1* involvement in plant response to Pi starvation.

P103

Transcriptome-wide exploring of yeast RNA secondary structure

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Recent advancements in RNA structure research, driven by the integration of chemical probing techniques, next-generation sequencing, and sophisticated bioinformatics, have shifted the perspective from studying individual RNAs to entire transcriptomes within cellular environments. Despite significant progress, the connection between mRNA structure, translation, and stability remains a topic of ongoing debate. Moreover, while the role of mRNA secondary structures in untranslated regions (UTRs) on gene expression has been extensively studied, the impact of coding sequence (CDS) structures on protein synthesis and mRNA stability remains less explored.

Here, we present the results of the first application of the SHAPE-MaP strategy on the eucaryotic transcriptome, Saccharomyces cerevisiae, both in vivo and under cell-free conditions. Our high-quality structural data explains the differences between mRNA folding in the presence and absence of cellular factors. We show that mRNA coding sequences in yeast exhibit greater accessibility to SHAPE modifications in vivo, indicating increased structural flexibility. Additionally, we investigated structural differences in CDS regions, 5' and 3' UTRs, discovering distinct structural features in these regions. To specify the impact of active cellular machinery on RNA folding, we conducted SHAPE-MaP experiments under stress conditions, like glucose starvation and cell growth inhibition. Our data show the global stabilization of CDS structure upon stress induction, similar to that determined under ex vivo conditions. Going further, we correlated the architecture of transcripts with the different functions performed by their protein products. We focused on the analysis of RNA structural data with information about their stability and translation efficiency to more precisely investigate the structure-function relationships during the RNA journey in the cell. This global structural characterization of yeast mRNAs provides a more precise picture of structure-mediated regulation of mRNA function.

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Expanding the human mitochondrial proteome via the non-canonical translation mechanisms

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Although mitochondria possess their genome, the vast majority of the mitochondrial proteins are nuclear-encoded. The best-characterised import strategy is via Mitochondrial Targeting Signal (MTS) on the protein N-terminus. However, the actual number of annotated human mitochondrial proteins is likely underestimated. Studies in yeast revealed that many non-mitochondrial proteins gain the MTS as the N-terminally extended isoforms (NTEs). NTE-proetoforms arise through noncanonical translation, particularly as a consequence of alternative (non-AUG) translation initiation sites (aTISs). These potentially dual localised proteins with cytoplasmic or nuclear main isoform and the alternative one targeted to the mitochondria, represent the so-called 'dark mitoproteome'. Due to their typically lower abundance, such mitochondrial proteoforms often evade detection by conventional proteomic or imaging approaches. To investigate this phenomenon in humans, we analysed translatomic data (RiboSeq) to identify NTE-isoforms. Our analysis uncovered 1678 unique genes, which corresponded to 3921 proteoforms, and among them, 1565 genes (3631 proteoforms) were not previously annotated as mitochondrial. Using the MitoProt II prediction tool, we assessed whether the NTEs gained or lost the potential for mitochondrial targeting. Among these, 2475 proteoforms gained and 1442 lost the predicted MTS as a result of N-terminal extension. Gene Ontology analysis of the MTS-gaining group revealed significant enrichment for RNA- and DNA-associated functions, mirroring findings in yeast. From this group, we prioritised candidates with high mitochondrial targeting probability that will undergo experimental validation.

P105

Exploring CAG Repeats in Non-Coding RNAs: Characteristics and Potential Impact on Neurodegeneration

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Trinucleotide repeats are present in many expressed sequences, including protein-coding and non-coding RNAs (ncRNAs). A specific feature of these tracts is their length polymorphism in the population, as well as the potential to cause diseases when expanded. CAG repeats have been associated with brain function, and mutations of these sequences in specific genes are responsible for neurodegenerative disorders.

In this study, we focused on RNAs with at least 10 CAG repeats, because such sequences are more likely to have a variable length in the population and/or to fulfill a functional role in the molecule. We identified human RNAs containing at least 10 CAG repeats in the reference datasets: 146 protein-coding transcripts, 28 long non-coding RNAs (lncRNAs), and 103 circular RNAs (circRNAs). The identified lncRNA and circRNA sequences originated from 10 and 20 different genomic loci, respectively.

Most of the repeat tracts in circRNAs were derived from mRNA exonic sequences. For several of them, we verified the back splicing junction sequence and circRNA expression in human brain and liver tissue. We also predicted the effect of potential repeat tract expansion on the structure of selected circRNAs.

For the selected *ATXN7* locus, we identified 9 circRNAs that contained a CAG repeat tract. We investigated the potential of these circRNAs (interestingly, containing a binding site for the NF-kappaB p65 subunit) to be implicated in pathological pathways in spinocerebellar ataxia type 7 (SCA7). SCA7 is caused by the expansion of the CAG tract in the *ATXN7* gene encoding the ataxin-7 protein, which contains an abnormal polyglutamine tract when mutated. Our preliminary results suggest that circRNAs derived from the ATXN7 locus and containing the repeat tract should be investigated in more detail in the context of disrupted pathways in SCA7.

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P106

Allele-selective therapeutic approach for Huntington's disease using artificial miRNAs (amiRNAs)

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Huntington's disease (HD) and other disorders caused by CAG repeat expansions belong to a group of uncurable neurodegenerative diseases. One of the most promising therapeutic strategies involves usage of the natural mechanism of RNA interference (RNAi). However, a key challenge in this approach lies in balancing therapeutic efficacy with safety, particularly with regard to minimizing off target effects. A previously developed artificial microRNA, amiR136-A2, targeting CAG repeats and containing a single mismatch at position 8 of the guide strand, demonstrated good *HTT* silencing efficiency and allele-selectivity. Nevertheless, its further development was limited by certain drawbacks, such as reduced activity in the cerebral cortex and the presence of fully complementary off target sequences.

The aim of this doctoral dissertation was to improve the previously designed amiR136 A2 by introducing an additional nucleotide substitution to enhance safety while maintaining or improving efficiency. Among the variants tested, the molecule amiR136-13A, featuring an extra mismatch at position 13, showed increased silencing efficiency against mutant *HTT* in HD cell models and *in vivo*, achieving a comparable therapeutic effect at half the AAV5 vector dose compared to the original construct.

amiR136-13A exhibited a reduced number of potential off-target sequences, a favorable cellular processing profile, increased guide strand release compared to amiR136-A2, and high 5'-end homogeneity in the striatum of YAC128 mice. In the in vivo model, the molecule was well tolerated up to 28 weeks post-injection, did not trigger microglial or astroglial activation, did not increase serum levels of neurofilament light polypeptide (NfL), and demonstrated no off-target effects.

Furthermore, in a cell model with inducible *HTT1a* expression, amiR136-13A effectively reduced levels of the toxic, truncated huntingtin isoform, which is known to significantly contribute to HD pathogenesis.

Taken together, the results indicate that amiR136-13A is a safer and more effective alternative to amiR136-A2, and represents a promising candidate for further development as a potential RNAi based therapeutic tool for HD and other polyglutamine disorders.

P107

MBNL splicing factors regulate the microtranscriptome of skeletal muscles

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Muscleblind-like proteins (MBNLs) regulate various RNA-processing steps, including alternative splicing, polyadenylation, RNA stability, and mRNA intracellular localization. In myotonic dystrophy type 1 (DM1), the most common muscular dystrophy in adults, MBNLs are sequestered on toxic RNA containing expanded CUG repeats, which leads to disruption of MBNL-regulated processes and disease features of DM1. We showed the significance of MBNLs in the regulation of microtranscriptome dynamics during postnatal development of skeletal muscles and in microRNA (miRNA) misregulation observed in mouse models and patients with DM1. We identified multiple miRNAs sensitive to insufficiency of MBNL proteins and revealed that many of them were postnatally regulated, which was correlated with increases in the activity of these proteins during this process. In adult Mbnl1-knockout mice, miRNA expression exhibited an adult-to-newborn shift. We identified two mechanisms through which MBNLs influence miRNA levels. First, MBNL loss induces transcriptional changes in miRNA precursors. Second, MBNLs affect miRNA biogenesis by regulating the alternative splicing of miRNA primary transcripts. We propose that the expression of miR-23b, miR-27b and miR-24-1, produced from the same cluster, depends on the MBNL-sensitive inclusion of alternative exons containing miRNA sequences. Our findings suggest that MBNL sequestration in DM1 is partially responsible for altered miRNA activity. This study provides new insights into the biological roles and functions of MBNL proteins as regulators of miRNA expression in skeletal muscles.

P108

Direct RNA sequencing of the mitochondrial transcriptome of *Candida albicans*: a proof of concept for organellar transcriptome analysis

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Organellar transcriptomes, though essential for cellular function, still remain relatively understudied, particularly with respect to full-length transcripts and posttranscriptional modifications. Direct RNA sequencing is a novel and powerful tool to overcome the limitations of second-generation RNA-seq platforms, enabling full-length isoform analysis and modification detection. However, there are still relatively few projects employing this method specifically for studying organellar transcriptomes.

In this study, we applied Nanopore direct long-read sequencing to profile the mitochondrial transcriptome of *Candida albicans*, a promising model for investigating nucleo-mitochondrial interactions. Our custom data analysis pipeline allowed for efficient mapping and detection of complete transcript isoforms as well as posttranscriptional RNA modifications in wild-type *C. albicans* and deletion strains in genes coding for the 5' and 3' mitochondrial RNA exonucleases CaPET127 and CaDSS1. Our findings allowed us to refine the 3' and 5' transcript boundaries from previous studies, as well as uncover intermediate splicing isoforms and changes in isoform abundances due to exonucleolytic processing disruptions. Additionally, multiple putative posttranscriptional modification sites were identified.

This work underscores the potential of direct RNA sequencing for comprehensive mitochondrial transcriptome analysis, offering novel insights into the *C. albicans* mitochondrial transcritptome and laying the groundwork for similar studies in other organisms.

P109

KH domains-mediated association of N4BP1 with the mRNA decapping machinery

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N4BP1 (Nedd4 Binding Protein 1) is a ribonuclease composed of two K-homology (KH) domains, a Ubiquitin Associated (UBA) domain, a NYN (N4BP1, YacP-like Nuclease) domain, and a CoCUN (Cousin of CUBAN) domain. It plays an important role in modulating immunological responses, NFκB signal transduction, and viral mRNA degradation. In this study, we demonstrate that N4BP1 interacts with EDC4, a key scaffolding protein of the mRNA decapping complex, as well as other proteins associated with 5'-cap hydrolysis in processing (P) bodies, including DCP1A, DCP2, and XRN1. To elucidate the molecular basis of the N4BP1-EDC4 interaction, we identified the two tandem KH domains (KH-1 and KH-2) as essential for this interaction. Mutational analysis and domain deletion studies revealed that the interaction between N4BP1 and EDC4 was disrupted upon deletion of the KH domains, mutations in KH-1 and KH-2 domains GxxG loops or RNase treatment, implicating an RNA-mediated mechanism. Moreover, we showed that the interaction of N4BP1 with EDC4 is necessary for the association with other decapping factors. Interestingly, both wild-type N4BP1 and KH domains mutants caused a reduction in HIV-1 mRNA levels, suggesting that N4BP1's RNase activity may not depend on its localization to P-bodies. In conclusion, our findings highlight the critical role of RNA in mediating the interaction between N4BP1 and EDC4. This study provides valuable insights into N4BP1's new functions within RNA-protein complexes and its involvement in mRNA decay pathways.

P110

FISHing for transcription termination regulators

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The transcription cycle consists of initiation, elongation and termination. Transcription termination punctuates genes, allows the release of nascent RNA as well as recycling of the engaged RNA Polymerase II (Pol II), and controls the expression of non-coding RNA. Similarly to the earlier phases in the cycle, correct transcription termination is required for proper gene expression. Despite it's importance, termination is still understudied and poorly understood.

Up to date, methods used to study termination have been mainly based on isolating RNA from cells and analyzing it in bulk from a cell population – either on a single gene level, or with the use of various sequencing methods. However, we are missing an understanding what is happening on a single cell level. Furthermore, we would like to understand better how transcription termination is regulated.

On my poster I will show my progress in addressing the two points raised above, by applying single molecule inexpensive RNA FISH (smiRNA FISH) to study both correct and read-through transcription termination. We are also performing a chemical screen for novel regulators of transcription termination with the use of this methodology.

P111

Structure-informed modulation of pathogenic alternative 3' splice sites

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Numerous pathogenic AG-creating mutations result in the formation of tandem alternative acceptor splice sites (TASS). By inducing frameshifts, introducing function-disrupting amino acids, or encoding premature termination codons, usage of mutant proximal acceptor sites is associated with diseases, including metabolic disorders, muscular dystrophies, immunodeficiencies, and cancer (Zhang et al., 2023). In all such cases, shifting splicing towards the canonical, distal 3' splice site offers a promising therapeutic strategy. However, this approach has remained unexplored due to limited mechanistic and structural understanding of TASS regulation, which was only recently shown to occur during the second catalytic step of splicing (Dybkov & Preußner et al., 2023). Here, we combine cryo-EM structural analysis, systematic RNA-Seq, machine learning, and functional in vitro splicing and minigene assays to uncover both cis- and trans-codes that determine TASS usage. We present a cryo-EM structure of the human P complex assembled on a beta-globin pre-mRNA, which reveals the complete architecture of the second step spliceosome active site. Our data show how the C-terminus of the C*-complex specific FAM32A protein is positioned in the active site by the evolutionarily ancient Prp18 protein, and uncover essential roles for the SDE2 N-terminal lysine and the Prp22 C-terminus in stabilizing the active second step conformation of the spliceosome. Importantly, structure-guided mutations in each of these second-step specific splicing factors are sufficient to redirect 3' splice site choice. In parallel, machine learning–driven mutagenesis of minigenes reveals when 3' splice site selection is determined primarily by cis-regulatory elements, and when trans-acting factors are required. We are now applying these insights to pathogenic variants, identifying those that are particularly responsive to modulation of specific regulatory factors. In selected cases, we find that restoring canonical distal splice site usage is achievable through targeted changes in trans-acting proteins. Altogether, we present a mechanistic and structural framework for TASS selection, with direct implications for therapeutic correction of over 200 disease-associated mutations.

P112

Beyond NMD: UPF1 Regulates R-loop Homeostasis During Transcription Elongation

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UPF1, a conserved RNA helicase and core effector of nonsense-mediated mRNA decay (NMD), plays key roles in post-transcriptional gene regulation. UPF1 loss is lethal in most eukaryotes, restricting in-depth study of its non-canonical molecular and biological functions. In *Arabidopsis thaliana*, we demonstrate that this lethality primarily arises from autoimmunity. By suppressing immune signalling in *eds1* or *pad4* backgrounds, we generated viable *upf1* null mutants. The *upf1 eds1* plants exhibit developmental phenotypes such as slow growth, delayed flowering, reduced vigour, and partial sterility. However, they consistently display better overall growth than *upf1 pad4* plants. This observation aligns with our transcriptome data, which indicates stronger suppression of immune response in the *eds1* background. Importantly, the reduced immune activation in *upf1 eds1* plants minimises secondary transcriptomic changes, enabling clearer identification and analysis of direct NMD targets as well as revealing UPF1 functions beyond canonical NMD.

To dissect UPF1's non-canonical functions, we generated helicase- and ATPase-deficient mutants in the *eds1* background. While both are similarly impaired in NMD, they exhibit distinct growth phenotypes, with the ATPase mutant growing slower than the helicase mutant, suggesting additional non-NMD functional differences.UPF1-GFP localisation reveals that the protein is predominantly cytoplasmic but accumulates significantly in the nucleus following Leptomycin B treatment, which inhibits nuclear export. This observation supports active nucleo-cytoplasmic shuttling of UPF1 and implicates potential nuclear functions beyond its established cytoplasmic role.

Given UPF1's nuclear localisation, genome instability signatures, and growth defects in mutants, we hypothesised that UPF1 may function in R-loop metabolism. Indeed, our strand-specific R-loop profiling (ssDRIP-seq) in upf1 eds1 uncovers significant R-loop accumulation along gene bodies, suggesting that UPF1 might suppress co-transcriptional R-loops during elongation. Together, these findings reveal a novel nuclear function of UPF1 in R-loop homeostasis, distinct from its canonical NMD activity. The upf1 eds1 background provides a powerful genetic system to dissect UPF1's essential and multifaceted roles in RNA biogenesis, without confounding influences from immune activation.

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P113

Regulation of auxin tropic response by alternative splicing

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The functional aspects of plant transcriptomes are highly unknown. Very little is known about the specific pathways regulating particular splice events towards determined developmental outputs. Auxin is a vital regulator of plant development which integrates the stimuli of changing plant environment. We have previously shown how two splice isoforms of the major auxin transporter, termed PIN7a and PIN7b, counteract on the plasma membrane to tune the tropic response towards light and gravity stimuli. Following up this project, we identified potential regulators upstream of this event. Our candidate approach lists a group of spliceosomal components and also factors involved in RNA methylation. They appear to be physiologically relevant regulators of the PIN7 splice site choice. Specifically, the tropic response of relevant knockouts of RNA processing factors matches the perturbed PIN7a/b expression ratio. We aim at describing the networks specifically co-regulated with AS of PIN7, and also search for the external cues which regulate this process.

P114

Global effects on RNA polii pausing, cleavage & termination on controlled rapid depeltion of human PCF11

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The complex life of an mRNA begins with 'transcription'. Through a well-coordinated multi-step pathway genetic information is unidirectionally transmitted from DNA to RNA. Although studied over decades, the understanding of this massive operation and all its participating factors remains incomplete. The most elusive being transcription termination and 3'-end processing. Through this work we attempt to shed light on the cross-talk between these closely connected yet mutually independent regulatory events shaping the 3'-end of genes from the perspective of one such interesting factor PCF11.

PCF11 is an essential autoregulatory protein with multifaceted functions along the RNA biogenesis pathway. Not only is it an integral component of mammalian cleavage factor II (CFIIm), but it also plays an important role in transcription termination and mRNA export. Yet another newly discovered function of PCF11 is its ability to prematurely terminate a subset of genes in human cells and during zebrafish development. The genes are mainly enriched for gene expression regulators.

While substantial correlative evidence supports the involvement of PCF11 in these processes, a detailed mechanistic understanding of the contribution of this protein remains unresolved. By employing rapid and controlled depletion of this critical regulator, we aim to elucidate how its diverse functions impact nascent mRNA biogenesis by influencing global transcriptional profiles. This approach will provide valuable mechanistic insights into the molecular role of PCF11 and its contribution to transcriptional regulation.

P115

Roles of natural antisense transcripts in breast cancer epitranscriptomics

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Breast cancer remains one of the most common malignancies among women globally, with its classification into subtypes driven by distinct molecular mechanisms. In our study, we focus on two subtypes: estrogen receptor-positive (ER+) breast cancer and the more aggressive triple-negative breast cancer (TNBC). Our aim is to explore the role of A-to-I RNA editing, a process dysregulated in cancer cells that diversifies the transcriptome by converting adenosines to inosines, resembling point mutations. This epitranscriptomic modification is catalysed by adenosine deaminase acting on RNA (ADAR) enzymes and depends on double-stranded RNA (dsRNA) structures as substrates.

We particularly investigate natural antisense transcripts (NATs), a class of long non-coding RNAs (lncRNAs) transcribed from the antisense strand of protein-coding genes. Their overlapping regions with sense transcripts can form dsRNA structures, potentially serving as ADAR substrates. Our study examines the contribution of NATs to A-to-I RNA editing through such dsRNA formations.

Using the SNP-free RNA editing site identification toolkit (SPRINT), we analysed RNA-seq data from two breast cancer subtypes ER+ (estrogen receptor positive) and TNBC (triple negative breast cancer) to identify candidate gene pairs. We discovered a previously unannotated NAT, *UGGT1-AS1*, and confirmed A-to-I editing events in its sense partner, *UGGT1*, by Sanger sequencing. RAP-RNA experiments validated the presence of dsRNA structures between these transcripts. Furthermore, knockdown experiments of *UGGT1-AS1* affected expression levels of *UGGT1* and influenced its stability *UGGT1*. Both transcripts were found predominantly localized in the cell nucleus. Notably, depletion of *UGGT1-AS1* affected cell proliferation and stability of *UGGT1* transcripts, emphasizing the functional importance of NATs in regulating gene expression. Our findings highlight the regulatory mechanism of novel natural antisense transcript *UGGT1-AS1* and provide new insights into epitranscriptomic regulation within breast cancer cells.

P116

Identification of novel factors and regulatory mechanisms controlling RNA in human mitochondria

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RNA degradation is known to play key roles in regulating the steady-state levels of functional RNAs and the removal of malformed or unnecessary transcripts.

The human mitochondrial genome encodes several types of RNAs, including mRNAs, tRNAs, rRNAs, and antisense non-coding RNAs (as-ncRNAs). Mitochondrial as-ncRNAs are synthesized at high levels. However, under normal conditions, they are kept at very low abundance. This indicates that as-ncRNAs are degraded very efficiently to (most likely) prevent interference with the expression of mRNAs or formation of double-stranded RNAs that may trigger sterile inflammation. In contrast, mitochondrial mRNAs are highly abundant in the cellular transcriptome and, although synthesized at same rates among themselves, display clearly different steady-state levels.

The processes of controlling mitochondrial antisense transcripts and differential expression of mt-mRNAs are essential for mitochondrial gene expression, yet they remain not fully understood. To investigate this, we aim to identify and characterize novel proteins involved in mt-asRNA degradation, as well as factors regulating the differential expression of mt-mRNAs. To this end, we performed a high-throughput screening using a custom siRNA library targeting 6.395 genes encoding proteins known or predicted to play roles in mitochondrial or RNA biology. Human cells were transfected with siRNAs, followed by measurement of mtRNAs with FISH to identify genes whose silencing affects the mitochondrial RNA levels. Qualitative and quantitative analyses of the screening data revealed some candidate genes whose silencing alters mitochondrial RNA expression. Here, we present our experimental strategy and some initial findings.

P117

Investigating the role of the neuroprotective RNA chaperone RBM3 in stress granules

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The RNA-binding motif protein 3 (RBM3) is known as a cold-shock protein strongly associated with the neuroprotective effects of hypothermia. Systemic cooling is used in some clinical settings for neuroprotection and RBM3 was suggested to be the main molecular mediator of its beneficial effects. Indeed, RBM3's induction restores memory, prevents synapse and neuronal loss, extends survival in preclinical mouse models of prion and Alzheimer's disease (Peretti et al., 2015, 2021), stimulates neurogenesis in rodent brain after hypoxic–ischemic brain injury (Zhu et al., 2019) and protects against neurotoxin effects in neuronal cell lines (Yang et al., 2019).Enhancing RBM3's expression using specific antisense oligonucleotides without cooling acts as neuroprotective in cellulo and in vivo and at the same time skips the risks linked to the therapeutic hypothermia, like blood clots and pneumonia (Preussner et al., 2023).

However, although the broad cytoprotective activity of RBM3 is well established, the mechanism of action that would explain the protective function remains poorly understood. Our data show that RBM3 is localized in stress granules, cytoplasmic mRNP assemblies that form in response to stress stimuli to maintain cellular homeostasis. Using RBM3 knock out cell lines and ASO-mediated increase of RBM3 expression, we show that RBM3 controls the number, size and dynamics of stress granules. Furthermore, RBM3 controls the mRNA content of stress granules, suggesting a global role in mRNA metabolism in stress conditions. As all disease conditions that benefit from increased RBM3 expression are associated with cellular stress, a function of RBM3 in controlling stress granule biology provides a unifying molecular mechanism to explain its broadly cytoprotective role. Understanding the molecular function of RBM3, and addressing pathways through which RBM3 controls stress granule biology, will contribute to further development of therapeutic concepts based on increasing RBM3 expression for combating neurodegenerative diseases and other stress-related disorders.

P118

Rearrangements of the spliceosomal RNA core after the first step of splicing

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The catalytic triplex is an important component of the RNA catalytic core, first identified in group II intron structures (Toor et al., 2008) and later confirmed in spliceosomes by genetic analysis (Fica et al., 2014). Intriguingly, all cryo-EM structures of catalytic spliceosomes reveal unchanging structures of the catalytic triplex found at different stages of splicing. By contrast, group IIB intron structures detect two distinct conformations formed after the first step (Chan et al., 2018), suggesting that the catalytic triplex must change after the first step. Using genetic analysis in yeast , we asked if analogous rearrangements occur during the spliceosomal process. Group IIB models predict that only one of the three triples of the first step conformation, 1-3, rearranges to form two new transition state triples, T-3 and T-4, which then change again to form a distinct 2-3 second step triple.

We tested all possible nucleotide combinations of the first step 1-3 triple (extending the earlier analysis of Mefford and Staley, 2009), and of T3, T4, and 2-3 triples, predicted to form after the first step. Most of the resulting mutant combinations are inviable, and even most of the viable ones display significant splicing defects. We tested the effects of other spliceosomal alleles known to affect a particular conformation (i.e. alleles stabilizing the first or the second step conformation or U6 mutants facilitating exit from the first step) on various triples, analyzing their growth phenotypes and splicing defects.

Our results fully support rearrangements of the catalytic triplex described for group IIB introns. Upon exit from the first step, two distinct T3 and T4 triples of the transient transition state conformation are formed, facilitating the reposition of substrates for the second step. Subsequently, the catalytic core adopts the second step catalytic conformation, similar to, but distinct from, that of the first step. These dynamic rearrangements at the RNA core may affect alternative splicing pathways.

P119

Toward Understanding the Developmental Tuning of RNA Polymerase II Elongation Speed

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Neurodevelopment relies on a finely balanced interplay between neural stem cells (NSCs) self-renewal and lineage commitment. However, the contribution of RNA polymerase II (RNAPII) elongation kinetics to this process remains largely unexplored. We previously showed that a mouse model of slowly elongating ("slow") RNAPII (*Polr2a R749H*) is lethal, and that NSCs derived *in vitro* from RNAPII R749H-expressing embryonic stem cells (ESCs) fail to self-renew and show signs of terminal differentiation. Based on these findings, we hypothesized that precise control of RNAPII transcriptional speed is critical for orchestrating gene expression programs that drive neural differentiation. To investigate the mechanisms regulating RNAPII elongation rate during neurodevelopment, we employed an *in vitro* differentiation model, in which pluripotent ESCs first exit the naïve state and are then directed to differentiate into NSCs.

First, to measure RNAPII kinetics changes during differentiation, we used time-resolved nascent transcript sequencing; 5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside / Transient Transcriptome sequencing (DRB/TT-seq) on ESCs, cells that exited pluripotency, and NSCs. In this method, cells are treated with DRB to arrest RNA polymerase II (RNAPII). Upon DRB washout, RNAPII molecules are synchronously released from promoters. Labelling nascent RNA with 4-thiouridine (4sU) followed by sequencing enables analysis of RNAPII progression along gene bodies and thus elongation rate estimation. This analysis revealed that NSCs exhibit significantly accelerated RNAPII progression compared to ESCs, suggesting stage-specific tuning of transcription kinetics.

Further, to identify regulators of RNAPII speed, we isolated chromatin-bound RNAPII complexes from ESCs, cells that exited pluripotency, and NSCs using antibodies against unphosphorylated and phosphorylated RNAPII, followed by label-free mass spectrometry. We identified RNAPII-associated interactome across developmental stages. Differential interactors will be validated to uncover potential regulators of elongation kinetics during neurodevelopment. We anticipate that this will provide insight into how RNAPII elongation is controlled by different factors during neurodevelopment.

P120

Canonical cap footprint in Arabidopsis

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mRNA decapping is conserved and essential mechanism in post-transcriptional gene expression regulation in eukaryotes, acting as a key determinant of transcript stability and turnover. The decapping enzyme DCP2 removes the protective 5' cap structure, thereby committing the transcripts to subsequent degradation. Despite its evolutionary conservation across diverse taxa including fungi, animals, and plants, we still do not fully understand all of its substrates and its physiological implications. In this study, we provide a comprehensive analysis of DCP2-dependent mRNA cap footprints within *Arabidopsis thaliana* seedlings to identify the targets and functions associated with canonical decapping in plants.

Here, we used a multifaceted approach that combines *in vitro* DCP2 treatment with degradome sequencing and transcriptome profiling. Using the methodology, we mapped over 13,000 high-confidence capped transcripts, and identified distinct cap signatures in both wild-type (WT) and *dcp2* null mutants. As expected, the majority of 5' caps were situated in proximity to annotated transcription start sites (TSSs), particularly within promoter regions, 5' untranslated regions (UTRs), or first exons. Importantly, the genes that were highly expressed in *dcp2* mutants displayed unique cap footprints, suggesting fast turnover in WT while increased stabilization in absence of decapping activity. Among *dcp2* unique caps, we discovered capped transcripts originating from previously unannotated loci located in intergenic regions of the genome. This suggests that DCP2 play a role in regulating transcripts from these regions, which otherwise might be rapidly degraded in WT via decapping. Furthermore, we identified a subset of genes containing multiple 5' caps, a feature that is prevalent in *dcp2* mutants than WT. This highlights the role of decapping in clearing unwanted transcripts. Clearly, there is transcript heterogeneity and the generation or stabilization of more than one transcript per gene in decapping-deficient context. Among the previously identified high-confidence nonsense-mediated decay (NMD) targets, many targets were capped and unique to *dcp2* mutants. We provide a direct evidence that NMD substrates are degraded via canonical DCP2-dependent decapping.

Collectively, our results elucidate that DCP2-mediated decapping is crucial in maintaining transcriptome homeostasis. This study provides a valuable resource for understanding DCP2 substrates and highlights the functional significance of decapping in modulating gene expression networks.

P121

Designing artificial xrRNA structures for the regulation of gene expression

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Structured elements within viral RNA genomes play a vital role in the life cycle of RNA viruses. A specific family of viral RNA elements, called exoribonuclease-resistant RNAs (xrRNAs), is located in the 3' untranslated region of the RNA genome of Flaviviruses, like the prominent pathogenic Zika and Dengue viruses. This specialized structure protects downstream RNA sequences from degradation by 5'-3'-exoribonucleases, therefore facilitating resistance to host cell defenses. The mechanism of this protection relies on the three-dimensional fold of the xrRNA, which features a distinctive ring-like structure that wraps around the 5' end, from where the exonuclease approaches. This effectively blocks the progression of exoribonucleases like XRN1 through purely mechanical interactions, extending the half-life of downstream sequences independent of any protein involvement. This makes xrRNAs suitable as a potential building block for RNA-based therapeutics and synthetic biology.

To explore the use of xrRNAs in novel applications, a deeper understanding of the structure-function relationship is required. Therefore, we modified structural elements of biological xrRNAs using a rational approach. We then used the resulting knowledge to design new and unique sequences which can replicate fold and function of xrRNAs found in nature. Our designs are first compared to biological xrRNAs in silico, using molecular dynamics simulations to approximate the effect of XRN1, and then experimentally verified and characterized through XRN1 degradation assays and structure probing. We show that such designs can be reliably constructed and tuned to different levels of resistance.

P122

Pipeline for identification of a collection of *bona-fide* interactors of the uncharacterized protein TTC33, which is relevant to DNA damage response

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About 10% of human protein-coding genes are uncharacterized. This is a significant gap in our understanding of cell physiology. Many such proteins are purely structural, meaning that their function is hard to predict as it cannot be simply inferred based on the presumed enzymatic activity. Mass spectrometry (MS) is often used as the first step in characterizing interactors of such proteins. However, MS is a sensitive method, known to produce false-positives. To counter this issue in this work, I present a pipeline rooted in label-free MS (LF-MS), that cross-compares proteins significantly co-purified with the bait of interest from at least two cell lines, ultimately giving a workable list of interactors. We developed this approach to characterize the human 30 kDa structural TTC33 protein composed of tetratricopeptide repeats, and conserved only in bony vertebrates. We thus identified a TTC33-associated network of interactions (TAN) composed of uncharacterized coiled-coil protein CCDC97, WDR61 (SKIc and PAFc subunit), PP2A-B55α phosphatase (PPP2R1A, PPP2R2A, PPP2CA/CB), UNG1/2 uracil DNA glycosylase, which initiates Base Excision Repair, PHF5A and other SF3A/B subunits of this U2-subcomplex. Reverse LF-MS analysis of WDR61 and CCDC97 interactors largely confirmed this list, but did also reveal possible mutually exclusive interactions between TTC33 and UNG1/2 or CCDC97 Altdough TTC33 interacting with WDR61 and PHF5A – proteins known to participate in RNA metabolism – our RNA-seq analyses showed only minimal changes on gene expression or splicing outcomes. We next explored the concept of function by proximity, and hypothesized that similarly to UNG1/2 and PP2A also TTC33 could play a part in DNA damage response. We further show that depletion or loss of TTC33 and CCDC97 are associated with accumulation of DNA damage marker, H2AXγ, which indicates increased DNA damage. Importantly a similar phenotype was observed in cells treated with doxorubicine, which introduced double-strand breaks. In sum, our high-throughput approach delivered a *bona-fide* list of interactors for TTC33, which was essential for phenotypic characterization. We intend to employ this method to systematically characterize other human structural proteins.

P123

Studies on the Oxidative Damage of the Wobble 5-Methylcarboxymethyl-2-Thiouridine in the tRNA of Eukaryotic Cells with Disturbed Homeostasis of the Antioxidant System

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We have previously shown that 2-thiouridine (S2U), either as a single nucleoside or as an element of RNA chain, is effectively desulfurized under applied in vitro oxidative conditions. The chemically induced desulfuration of S2U resulted in two products: 4-pyrimidinone nucleoside (H2U) and uridine (U). Recently, we investigated whether the desulfuration of S2U is a natural process that also occurs in the cells exposed to oxidative stress or whether it only occurs in the test tube during chemical reactions with oxidants at high concentrations (1). Using different types of eukaryotic cells, such as baker's yeast, human cancer cells, or modified HEK293 cells with an impaired antioxidant system, we confirmed that 5-substituted 2-thiouridines are oxidatively desulfurized in the wobble position of the anticodon of some tRNAs. The quantitative LC-MS/MS-MRMhr analysis of the nucleoside mixtures obtained from the hydrolyzed tRNA revealed the presence of the desulfuration products of mcm5S2U: mcm5H2U and mcm5U modifications. We also observed some amounts of immature cm5S2U, cm5H2U and cm5U products, which may have indicated a disruption of the enzymatic modification pathway at the C5 position of 2-thiouridine. The observed process, which was triggered by oxidative stress in the living cells, could impair the function of 2-thiouridine-containing tRNAs and alter the translation of genetic information.

(1) Sierant et al. Int J Mol Sci. 2024 Nov 17;25(22):12336. doi: 10.3390/ijms252212336

P124

MpSPL3 gene is indispensable for generative organs development in Marchantia polymorpha

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SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes encode plant-specific transcription factors that are widely distributed across the plant kingdom. In angiosperms, the multimember *SPL* family regulates different biological processes, such as vegetative-to-reproductive phase transition, inflorescence architecture, and lateral organ development. In contrast, the liverwort *Marchantia polymorpha* genome encodes only four *SPLs*, with functional studies available only for microRNA-targeted members, Mp*SPL1* and Mp*SPL2*. While Mp*SPL1* was shown to control the meristem dormancy to modulate the thallus architecture, Mp*SPL2* was found to promote the transition from vegetative-to-reproductive phase. Here, we investigate the impact of the Mp*SPL3* gene on Marchantia development, an Mp*SPL* family member non-targeted by microRNA. We show that the Mp*SPL3* gene is crucial for the successful coordination of the vegetative growth and the reproductive phase transition. The knockout of Mp*SPL3* leads to strong growth retardation with disordered thallus branching, reduced gemma cup number, and, most profoundly, abolished gametangiophores production. Interestingly, overexpression of Mp*SPL3.2*, a shorter isoform, has no morphological effect, whereas the overexpression of Mp*SPL3.1*, a longer isoform, results in a delay in timing and reduced efficiency of gametangiophores formation. Moreover, Mp*SPL3* is responsible for direct or indirect expression control of the remaining Mp*SPL* family members as well as key genes involved in the germ cell specification, Mp*LRL* (*LOTUS JAPONICUS ROOTHAIRLESS-LIKE*) and Mp*CKI1* (*CYTOKININ-INDEPENDENT 1*), as the expression level of all these genes is significantly downregulated in the null Mpspl3 background. Altogether, our findings indicate that Mp*SPL3* plays a crucial role in regulating gametophyte development and reproductive success in Marchantia.

P125

Alteration of certain miRNA biogenesis in Burkitt lymphoma cells exposed to ionizing radiation

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Ionizing radiation (IR) is a key component of cancer therapy triggering oxidative stress responses. MicroRNAs (miRNAs) are non-coding RNAs that inhibit gene expression at the post-transcriptional level and may contribute to the radiosensitivity of cancer cells. Biogenesis of miRNAs includes transcription as long primary miRNAs (pri-miRNAs), that are processed into precursor miRNAs (pre-miRNAs), which are transported to the cytoplasm for further processing to mature miRNAs. An indication for a regulated miRNA biogenesis is a variable ratio between primary and mature miRNA levels.

In this study, we examined the effect of IR on miRNA biogenesis in Burkitt lymphoma (BL) cell lines, CA46, DG75 and ST486. We observed induced apoptosis and cell cycle arrest in the G2/M phase after exposure to 4Gy of IR of all 3 BL cell lines. To profile miRNAs and pri-miRNAs, we performed RNA-seq and small RNA-seq in BL cells collected 4h and 12h post IR and non-IR control cells. Pri-miRNA levels were determined as the number of reads covering the pre-miRNAs with 150 nucleotides of flanking sequences. We showed more than1.5 fold induction in the levels of 4 miRNAs i.e. miR-146a, miR-449c, miR-449a and miR-155 after IR in at least in two BL cell lines. Next, we identified miRNAs with discrepancies in the miRNA to pri-miRNA ratios upon IR that may indicate regulated miRNA processing. We identified 186 pri-miRNAs that were not processed to mature miRNAs in any of the 3 cell lines. Additionally, we specified pri-miRNAs upregulated or downregulated upon IR with no change in miRNA levels. To further dissect miRNA processing kinetics, we quantified levels of pri-miRNA and mature miR-146a, miR-155, and miR-449a by qRT-PCR at 1, 4, 8, 12, and 24 hours post IR. Pri-miR-146a and pri-miR-155 levels increased within 1–4 hours post IR, preceding the increase in mature miRNA levels, suggesting IR-induced transcriptional activation. miR-449a displayed a rapid processing pattern, with undetectable pri-miRNA levels and a peak in mature miRNA expression at 8 hours.

In conclusion, our findings showed that IR affected the biogenesis miRNAs in BL cells, in particular miR-155, miR-146a and miR-449a.

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P126

CFIm-Complex-Driven Alternative Polyadenylation Controls mRNA Length, Stability, Localization and Protein output in colon and other cancers

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Alternative polyadenylation (APA) generates transcript isoforms with distinct 3' untranslated regions (3'UTRs), impacting mRNA stability, localization, and translation. The Cleavage Factor Im complex—CPSF5 (CFIm25), CPSF6 (CFIm68), and CPSF7 (CFIm59)—plays a central role in APA site selection. However, its role in spatial gene regulation and cancer remains poorly defined.

We performed siTOOLs-mediated knockdown of CPSF5, CPSF6, and CPSF7 in HCT116 (colon cancer) and HepG2 (liver cancer) cell lines. RNA-seq analysis revealed widespread 3'UTR shortening in CPSF5/6-depleted cells (2184 and 2095 genes, respectively), with smaller effect from CPSF7. APA-induced isoform shortening was largely uncoupled from changes in transcript abundance, supporting recent findings that mRNA length and abundance are orthogonal regulatory axes. Gene ontology analysis of APA-affected transcripts identified enrichment in cancer-relevant pathways, including p53 signaling, nucleocytoplasmic transport, and ribosome biogenesis.

Among the transcripts with shortened 3'UTRs, CD47 exhibited enhanced mRNA stability and striking mislocalization of its encoded protein—from the plasma membrane to the endoplasmic reticulum—following CPSF5/6 depletion, as well as after CD47 long 3’UTR isoform depletion. Confocal microscopy confirmed this redistribution, implicating APA in controlling spatial protein targeting. Additionally, ERK1/2 signaling was altered, with p-ERK condensates dissolving upon APA disruption. Other APA targets include NET1, a long-3'UTR isoform previously linked to apical-basal localization in colon epithelia.

To expand these findings, we are developing a method to isolate organelle-specific RNAs using antibody-based FACS sorting of cellular compartments (e.g., nucleus, ER, mitochondria, lysosomes, proteasome, TIS granules, plasma membrane, and cytosol), followed by isoform-resolved RNA-seq. This approach will enable subcellular mapping of 3'UTR isoform localization under native and CPSF-depleted (shortened 3’UTRs) conditions.

Our data reveal a conserved function for CPSF5/6 in regulating APA and mRNA localization across cancer cell types, suggesting that APA serves as a spatial code for correct protein targeting. These findings underscore APA’s therapeutic potential in modulating oncogenic protein distribution and cellular architecture in colon and other cancers

P127

Biogenesis from MIR155HG in B-Cell Lymphoma: A Modelling Approach

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The MIR155 Host Gene (MIR155HG) has three distinct molecular products: microRNA- miR-155, long non-coding RNA – lncRNA-155 (also known as BIC), and a micropeptide- miPEP155. While miR-155 has been implicated in B-cell lymphoma pathogenesis and is considered a promising therapeutic target, the roles and expression of lncRNA-155 and miPEP155 remain largely unexplored.

The goal of this project is to develop a mathematical model that provides a comprehensive view of how these products are generated and potentially influence each other's function in the context of B-cell lymphoma. We developed a preliminary linear model describing the interactions between the three MIR155HG products. The model includes five variables: the unspliced transcript, the spliced transcript (lncRNA-155) located in the nucleus and in the cytoplasm, mature miR-155, and the miPEP155. Based on this model structure, we identified key parameters that must be experimentally determined to improve modelling accuracy. To provide data for model calibration, we determined levels of miR-155, unspliced and spliced BIC transcript (lncRNA-155) in a panel of B-cell lymphoma cell lines and observed variable unspliced/spliced BIC transcript ratios. We also examined the impact of ionizing radiation on MIR155HG product levels in lymphoma cells by qRT-PCR and showed that total BIC transcript levels increased within 1–4h post-IR, preceding the rise in mature miR-155 at 12h, suggesting transcriptional activation of MIR155HG. We also observed upregulation of the spliced BIC (lncRNA-155), but not the unspliced BIC transcript.

In conclusion, we have developed a mathematical model that explores the interplay between MIR155HG-derived products and offers a framework to integrate biological observations, such as our finding that ionizing radiation modulates biogenesis from MIR155HG. Ongoing work includes follow-up biological experiments, validation and refinement of the model to better reflect the molecular dynamics underlying B-cell lymphoma.

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P128

Antisense oligonucleotides targeting viral RNA G-quadruplexes

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Viruses are important pathogens that exist worldwide and are present in humans, animals, plants, and other living organisms. Viruses with RNA genomes, such as influenza A virus (IAV), have the greatest pandemic potential of all the virus-related threats to humanity. It is known that the secondary structure of viral RNA (vRNA), including the IAV, is highly conserved among viral strains and biologically important during the viral life cycle. The RNA secondary and tertiary structures control many processes during viral replication. The exploration of novel antiviral approaches is of paramount importance to address the growing challenges posed by viral infections. Therefore, developing alternative therapeutic strategies by targeting structural motifs of vRNA (G-quadruplexes) represents a highly promising and innovative approach.

There is a growing interest in RNA secondary structures within the viral genomes. The presence of potential G-quadruplex-forming sequences (PQS) was confirmed in the genomes of RNA viruses, also in the IAV (by our group). The G-quadruplexes (G4s) are noncanonical structures that are formed within the G-rich sequences and are stabilized by Hoogsteen hydrogen bonds. Importantly, the G4s can play a crucial role in virus replication. Therefore, we postulate that G4 motifs can be considered a potential target for influenza infection.

According to our previous research, we identified a few G4 motifs within the vRNA of the influenza A/California/04/2009 (H1N1) using bioinformatics tools. We confirmed that three PQS fold into G-quadruplex structures by various biophysical methods. Based on the results, we designed a series of antisense oligonucleotides (ASOs) targeting G4 motifs. The unmodified and modified ASOs were chemically synthesized by the phosphoramidite approach on a solid support. The locked nucleic acid (LNA) and 2'-O-methylated modifications were introduced into ASO oligomers.

In the next step, we investigated the conformational changes of G-quadruplexes upon ASO-binding. To this end, the non-denaturing PAGE experiments were conducted. Electrophoresis investigations revealed that various ASOs can bind to the G4s and form complexes. Additionally, we are optimizing the fluorescence approach to get more insights into ASO-induced stabilization/destabilization. Finally, we expect that this study will allow us to select the most promising ASOs for further biological study.

P129

Searching For Factors Affecting CircRNA Dysregulation In Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) is a multisystemic disorder caused by expansion of CTG repeat in the 3' untranslated region (UTR) of the DMPK gene. The expanded CUG repeat RNA sequesters RNA-binding proteins like MBNL, leading to splicing defects, and alters the activity of CUGBP1 via upregulation, disrupting RNA processing. These changes contribute to the broad molecular pathology of DM1. While most studies have focused on linear splicing defects, recent findings suggest that circular RNAs (circRNAs), which are formed through back-splicing events, are also dysregulated and may contribute to the disease mechanism.

Several factors contribute to circRNA dysregulation in DM1. Using RNA-seq data from human *tibialis anterior* (TA), we observed that circRNA levels increase with CTG repeat size, with greater upregulation in samples harboring >1,000 repeats (DM1) compared to <90 repeats (proto-DM1). This was also observed in alternative splicing events, which were greater in DM1 compared to proto-DM1. Given that DMPK expression in mice is higher in TA-derived myotubes compared to TA tissue, we observed circRNA upregulation in myotubes relative to TA tissue in the DM1 Knock-In Mouse Model carrying 480 CTG repeats in both DMPK alleles. A similar trend was seen in alternative splicing events, with higher levels in myotubes than in TA.

Analysis of regulatory elements revealed that circRNA formation is influenced by some of the cis-acting factors, such as elongated flanking introns and the complementarity of their repetitive sequences (primarily *Alu* elements). However, the composition of dinucleotides and the circular back-splice junctions along with the strength of the splice sites do not appear to be major factors driving circRNA generation. Furthermore, trans-acting factors, including RNA-binding proteins, potentially modulate circRNA generation, contributing to their dysregulation in DM1.

P130

Single-template ligand-based methods for discovery of small-molecule nucleic acid binders

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Computational methods play a central role in the early stages of drug discovery and are widely used for virtual screening. Emerging discoveries about the role of RNA in disease have sparked growing interest in using RNA as a potential target for novel therapies. However, given the limited availability of experimentally determined 3D RNA structures and the insufficient accuracy of in silico RNA structure prediction methods such as AlphaFold 3, there is a significant demand for ligand-based methods that are independent of the target structure. We have benchmarked several such algorithms, which were primarily designed for proteins but can also be applied to RNA by their nature. Our results show that some ligand-based methods can effectively build predictive models for RNA targets, while the performance of others is similar to random selection.

P131

CRISPR-Cas9 technology-based excision of expanded CGG trinucleotide repeats from mutant *FMR1* gene

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Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) and Fragile X Syndrome (FXS) are two diseases caused by the expansion of CGG trinucleotide repeats in the 5'UTR region of the *FMR1* gene. They originate from a trinucleotide repeat expansion containing from 55 to over 200 CGG repeats (CGG^{exp}). In the case of FXTAS, this results in elevated mRNA levels, which contribute to the reduction of fragile X mental retardation protein (FMRP) production and to the formation of toxic RNA from CGG^{exp}, as well as a toxic protein resulting from non-canonical translation of CGG^{exp}. In the case of FXS, FMRP production is very inefficient and often does not occur at all due to promoter methylation.

Use of the CRISPR-Cas9 system to remove the CGG^{exp} sequence from the *FMR1* gene is considered as one of the possible therapeutic strategies in FXTAS and FXS. In an earlier project, we identified the six most effective sgRNAs targeting regions upstream and downstream of CGG^{exp}.

The aim of this research was the generation and evaluation of a series of genetic constructs for specific CGG^{exp} excision. Each contains two sgRNAs (targeting sequences upstream and downstream of CGG^{exp}) and their simultaneous expression is possible. This was achieved by using two independent transcription units regulated by two U6 promoters. Several of these double constructs were created, each encoding two gRNAs. Each also contain an independent transcription unit for overexpression of Cas9 recombinase. The next goal was the modification of these constructs by adding a mutation within the Cas9 sequence that inactivates one of its two catalytic domains and has nickase activity. This allows for more specific targeting of the CGG^{exp} locus, with fewer off-targets.

This experiment resulted in the successful generation of two variants of constructs: both with two U6 promoter-driven sgRNAs, but each with a different variant of Cas9. The generated constructs' efficiency was tested in HEK293 cells, as well as a HEK293 Flp-in line containing 95 CGG repeats. After transfection, it was determined that all wild-type Cas9 constructs very efficiently excised CGG^{exp}, while two Cas9 nickase constructs showed modest efficiency.

P132

Uncoupling of transcription termination and pre-mRNA 3’ processing during cancer progression

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Polyadenylation site (PAS) usage and the cellular machinery responsible is modified in many cancers. In particular, two key factors required for pre-mRNA 3’ cleavage and polyadenylation (CPA), CPSF73 and PCF11, have oncogenic properties – their elevated expression is associated with worse patient prognosis. Both proteins also function as transcription termination factors. Termination occurs over a thousand nucleotides downstream of PAS in humans. Our studies show transcription termination shifting proximally in colorectal cancer (CRC) cells. Earlier termination is driven by PCF11 redistribution on chromatin and linked to its protein levels. Notably, compared to cells from primary tumor, which are particularly sensitive to CPA levels, metastatic CRC cells showed partial reversal to termination patterns of normal cells. Surprisingly, changes in PAS usage and transcription termination during CRC are uncoupled. Thus, oncogenic properties of CPA factors are a result of increased proximity of pre-mRNA cleavage and transcription termination rather than alternative polyadenylation.

P133

HIF-1α mediated regulation of miRNA processing in adrenocortical cells

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Oxygen availability is a critical environmental factor necessary for survival. Aerobic organisms have evolved systems of sensing low oxygen pressure (hypoxia), which can occur physiologically or as a result of pathological conditions. The detection and downstream responses of cells to hypoxia occur mainly via the HIF (Hypoxia-Inducible Factor) signalling pathway. We have previously shown that HIF-1α transcription factor is crucial for regulating steroid synthesis in adrenocortical cells upon hypoxia (Watts et al., 2021; Cell Mol Life Sci.). Subsequently, we demonstrated that this modulation of hormone synthesis involves upregulation of the expression of several miRNAs targeting crucial enzymes involved in steroidogenesis in vitro and in vivo (Ariyeloye et al., 2025; Cell Commun Signal.). However, the mechanism by which HIF-1α regulates miRNA expression has not been fully understood.

In this study we used CUT&Tag to uncover the DNA binding profile of HIF-1α in Y1 adrenocortical cells subjected to a hypoxia-mimicking compound. Among these sites were genes for several members of the miRNA processing complexes including some of the protein constituents of the RISC complex. qPCR analysis of the expression of these genes was done in vitro in Y1 cells subjected to hypoxia or hypoxia-mimicking agents. Importantly, the RNA expression analysis was also conducted in adrenal gland extracts of mice subjected to hypoxia as well as in the HIF-1α KO animals. The results reveal a complex pattern of hypoxia and HIF-1α-dependent regulation of miRNA biosynthesis. In the future, a detailed analysis of the effects of HIF-1α-mediated modulation of these genes on miRNA processing will be conducted to elucidate the mechanism by which hypoxia controls miRNA function in steroidogenesis.

P134

MicroRNA Analysis of the Hepatoprotective Effect of Medicinal Herb Extracts Against Aflatoxin B1-Induced Hepatotoxicity in Porcine Model

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Aflatoxin B1 (AFB1), a potent hepatotoxin, compromises hepatic functionality by interfering with cellular mechanisms, including the regulation of RNA. Medicinal herbs like *Andrographis paniculata* (andrographolide), *Silybum marianum* (silymarin), and *Curcuma longa* (curcumin) exhibit hepatoprotective properties; however, the underlying molecular mechanisms—especially in terms of terms of **miRNA** modulation—remain underexplored. This research aims to analyze the regulatory dynamics of **miRNA** within the porcine hepatic transcriptome after exposure to AFB1 and to assess the hepatoprotective properties of herbal extract supplementation by applying next-generation genome sequencing (NGS) methodologies.

A total of 154 weaned piglets (aged 5-6 weeks) underwent the feeding experiment within a uniform environment with standardized experimental diets. The study was conducted in two stages: an initial pilot experiment consisting of 24 piglets, subsequently progressed to a larger-scale major experiments comprising 130 piglets, allocated across three distinct cohorts (36+44+50). The AFB1 experiment cohort specifically included 24 piglets. Phenotypic assessments, including liver function tests (LFTs), hematological/biochemical blood parameters, and fatty acid profiling were performed to find out the associations between molecular alterations and physiological responses. Total RNA was extracted from liver tissues of piglets exposed to AFB1-contaminated diets, with or without hepatoprotective herbal supplementation. RNA integrity (RIN >8) and purity (A260/A280: 1.8–2.0) were analyzed using an Agilent Bioanalyzer and NanoDrop 1000 spectrophotometer to ensure sample quality for subsequent procedures. Small RNA libraries

were prepared via Illumina adapter ligation, reverse transcription, and PCR amplification. Purified libraries quality assessment was performed using Agilent High Sensitivity DNA chips and sequenced on the Illumina MiSeq platform. To identify **miRNAs** that are associated with hepatoprotection, we will perform the differential expression genes (DEGs) analysis and weighted gene co-expression network analysis (WGCNA). Finally, functional annotation through gene ontology (GO) and pathway enrichment analyses will be carried out to clarify the molecular mechanisms that contribute to the protective effects of herbal supplementation.

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CFI polyadenylation factor – the beginning of the end?"

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All mature eukaryotic RNAs are processed from RNA polymerase II transcripts by capping, splicing (removal of introns and joining of exons), and 3'-end cleavage followed by poly(A) tail addition. Intron removal is catalyzed by the spliceosome, a complex of U1, U2, U4, U5, and U6 snRNPs and other proteins. In addition to its role in splicing, U1 has also been described as a key factor in inhibiting premature polyadenylation—a process known as telescripting. Since we had previously discovered a similar mechanism in *Arabidopsis thaliana* while studying introns in miRNA genes, we decided to investigate how the U1 snRNP interacts with the polyadenylation machinery in plants.

Our research identified a U1 snRNP protein network that included plant homologs of the human CFIm complex. Further transcriptome analysis of CFI mutants revealed its fundamental role in cleavage and polyadenylation (CPA) site selection at the global gene level. We also found that the CFI complex is involved in alternative polyadenylation within introns, suggesting a possible role in telescripting, as previously reported in human cells. However, our latest findings suggest that it is the CFI complex – not the U1 snRNP – that protects CPA sites from cleavage and polyadenylation. This property of the CFI complex is a key factor contributing to the heterogeneity of mRNA 3' ends in plants.

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Identification of novel barley (*Hordeum vulgare* (L.)) miRNAs involved in chloroplast development

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MicroRNAs (miRNAs) are short, single-stranded, noncoding RNAs, usually of 21 nt long. Their predominant role is posttranscriptional gene expression regulation. MiRNAs regulate the life cycle of plants, the formation of individual organs, and plant response to environmental stresses. Yet miRNAs related with chloroplast development are unknown. WHIRLY1 (WHY1) is a DNA/RNA binding protein localized to chloroplast and nucleus of barley cells. In Arabidopsis the WHY proteins were proven to protect the integrity of chloroplast genome. In this study we were interested in identification and quantitative analysis of the microtranscriptome of *why1* barley using Next Generation Sequencing (NGS) technology. Target mRNAs for the identified miRNAs were found in barley RNA degradomes using Parallel Analysis of RNA Ends and 5' RACE methods. Mapping of sRNA reads to barley genome followed by folding of the flanking sequences allowed us to identify 44 novel barley miRNA/miRNA* pairs. Selected novel miRNAs were confirmed by northern hybridization. *why1* barley chloroplasts are characterized by altered structure and delayed development. Among the miRNAs with increased accumulation in *why1* barley, was miRNA9662 (a molecule found only in monocotyledonous plants). Interestingly, the target mRNAs for miRNA9662 are Mitochondrial Transcription Termination Factor Family Proteins, mTERFs. mTERFs coordinate the expression of mitochondrial and chloroplast genes. Consistently, miRNA9662 is associated with the development of chloroplasts and mitochondria. Another example of identified module involved in plastid and mitochondria function is novel miRNA originating from cluster 27069 targeting pentatricopeptide repeat-containing proteins. The micromanagement of chloroplast development was not studied before, therefore our results shed new light to this field. Additionally, we have identified large set of novel barley miRNAs, which role in plants is yet to be studied.

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Novel proteins involved in the FUS-mediated biogenesis of sdRNAs in human cells

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The variety of non-coding RNAs is still growing, including an emerging class of small RNAs called sdRNA. These are stable RNAs 25-35 nucleotides long, derived from snoRNAs (small nucleolar RNAs), identified in animals, plants, fission yeast, and protozoa. They may function as microRNAs and regulate gene expression. However, the mechanism of sdRNAs generation in the cell is not yet fully understood.

Recently, in cells with FUS protein depletion (FUS KO) we have observed differential expression of numerous snoRNAs followed by an altered level of the corresponding sdRNAs, suggesting that FUS is involved in this process. FUS is a multifunctional protein involved in many pathways of RNA metabolism. It is localized mainly in the cell nucleus, however, there are mutations in the FUS gene that trap the FUS protein in cytoplasmic aggregates. These mutations have been associated with the neurodegenerative disease amyotrophic lateral sclerosis (called ALS-FUS hereafter). In cellular models of ALS-FUS, we have observed changes in the level of snoRNAs and sdRNAs as well.

To elucidate the molecular mechanism of FUS-mediated sdRNA biogenesis, we first performed RNA antisense purification (RAP) of selected snoRNAs from protein extracts of SH¬-SY5Y WT and FUS KO cells followed by mass spectrometry to identify snoRNA-interacting proteins. This resulted in the selection of four proteins: RALY, ILF3, DDX27, and DHX30, as potential candidates that participate in snoRNA biogenesis together with FUS. Next, we confirmed these interactions by IP (FUS:proteins) and tested the effect of FUS on these proteins expression (at the mRNA and protein level) by RT-qPCR and Western Blot. Moreover, we analyzed how the FUS:snoRNA and FUS:protein interactions were affected by ALS-FUS mutations, FUS P525L and FUS R495X using CRISPR-edited SH-SY5Y cells. Furthermore, due to the pathological cytoplasmic phenotype of FUS in ALS, we tested the subcellular localization of selected proteins by immunofluorescence.

The results obtained enable us to indicate a novel protein(s) that interact with FUS and participate in the processing of snoRNAs into sdRNAs. It will bring us closer to the mechanism of sdRNA biogenesis mediated by FUS and its role in the pathology of ALS.

P138

The role of the short non-coding vault RNAs and TEP1 protein in breast cancer cells response to treatment

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Vault RNAs are short non-coding RNAs transcribed by RNA polymerase III. Together with three proteins (TEP1, MVP, vPARP) they are components of the largest identified so far, eukaryotic ribonucleoprotein particles (RNPs), termed vaults. Interestingly, they are suggested to play a role in various processes including cellular differentiation, development, signal transduction, cellular transport, apoptosis, autophagy, proliferation, and drug resistance. Vault RNAs may alter cell metabolism via modulating target protein activity and function also in cancer cells. Consequently, they are considered potential prognostic marker/target in therapy.

Our study was performed on the panel of selected breast cancer cell lines MCF-7, MDA-MB-231, MDA-MB-453, MDA-MB-468, SK-BR-3, T-47D, and non-cancer breast cells (MCF-12A). Cell lines of different genotypes and phenotypes reflected diversity of breast cancer types that show different invasiveness, drug resistance, growth factor receptors, etc. First, the basal levels of four human vault RNA paralogs were evaluated (qPCR) in all studied breast cells. Cell showing the highest levels of selected vault RNAs were subjected to siRNA-mediated silencing of these RNAs. To evaluate the association between vtRNAs levels and studied cells' survival and response to therapeutic agents, evaluation of cell viability (MTT) and proliferation markers (PCNA, p21, p27, cyclin B1; western blot) after exposure to doxorubicin or cisplatin were performed. Furthermore, since one of the vault proteins, TEP1, is known as a RNA-binding protein, siRNA-mediated silencing of this protein was performed to check how it could impact vault RNAs levels and breast cancer cells response to drugs.

The study showed that in MDA-MB-231 cells, increased concentration of doxorubicin and longer treatment time correlated with increased expression levels of vault RNAs. Alterations in MVP, TEP1 and vPARP expression was also observed after doxorubicin or cisplatin treatment in MDA-MB-231, T47D and MCF12A cells. Furthermore, TEP1 silencing led to increased sensitivity of these cells to doxorubicin or cisplatin that was accompanied by altered proliferation and changes in the levels of proteins involved in cell cycle regulation. Thus we suggest that vault RNA paralogs contribute to the response of breast cancer cells to therapeutic agents and may be considered important players when designing anticancer strategy.

P139

Cis-Regulatory Element Within the MBNL1 Locus Modulates Tissue-Specific MBNL1 Isoform Diversity

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MBNL1 (Muscleblind-like 1) is a key regulator of alternative splicing, whose dysregulation underlies Myotonic Dystrophy type 1 (DM1) disease. While MBNL1 has been widely studied at the protein-coding level, the regulatory complexity at its 5' region remains underexplored. Here, we identify and characterize a cis-acting regulatory element located downstream of exon 1 of MBNL1. This element constitutes an alternative exon with intronic polyadenylation signal. Its inclusion leads to the production of a truncated but stable mRNA isoform, called MBNL1a, and attenuates the production of a full length MBNL1.

MBNL1a is expressed at low levels across most tissues but shows robust expression in spleen and thymus as well as in immune cells. In the latter, MBNL1a is expressed at levels comparable to the full-length isoform, and this expression originates predominantly from two MBNL1 transcription start sites. The transcript is polyadenylated, stable, and most likely exported to the cytoplasm.

Our data suggest that transcriptional dynamics influence exon 1c inclusion: overexpression of a slow RNA polymerase II mutant in HEK293 cells enhances MBNL1a production. Moreover, antisense oligonucleotide (ASO) screening identified a cis-regulatory region downstream MBNL1 exon 1 whose blockade increases alternative exon inclusion by up to 20-fold. This region overlaps a conserved binding site for a repressor-acting RNA-binding protein whose depletion robustly increases MBNL1a level.

Interestingly, MBNL1 itself may contribute to autoregulatory feedback, as its depletion increases MBNL1a levels, and ASO screening suggests MBNL1 binding. Functional studies indicate that the alternative exon is embedded within a cluster of potential proximal enhancers, and that a deletion of this regulatory element or a nearby putative enhancer through CRISPR-Cas9 modulates MBNL1 expression.

Collectively, our findings highlight a novel post-transcriptional regulatory mechanism controlling full-length MBNL1 expression, involving a cis-acting regulatory element and trans-acting protein factors that together establish a tissue-specific repertoire of MBNL1 isoforms.

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Modeling of mRNA deadenylation rates reveal a complex relationship between mRNA deadenylation and decay

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The polyadenosine tail (polyA-tail) is added to the mRNA 3'end co-transcriptionally to promote its export from the nucleus, and translatability in the cytoplasm. Export of coding transcripts is mediated by the Mex67-Mtr2 dimer, which if inactivated in Saccharomyces cerevisiae results in rapid degradation of the newly produced transcript, effectively cancelling synthesis. We took advantage of this phenomena to model cytoplasmic mRNA deadenylation and decay by producing large chase direct RNA sequencing datasets. This enabled numerical modeling of deadenylation using a modified gamma distribution, estimating the transcriptomic deadenylation rate at 10 A/min. Importantly, this entailed that the mRNA polyA-tail can be removed within minutes, which explained the estimated median transcript half-life of 9 min. A simplified independent method, based on the delineation of quantile polyA-tail values, further supported a correlation between the decay and deadenylation rates of individual mRNAs, which appeared consistent within some functional transcript groups and associated with codon optimality. We next inquired if the apparent functional link between deadenylation and decay was maintained during stress response. During the period of immediate adaptation to acute thermal pressure, cells rapidly switch off transcription of around 300 abundant mRNAs, including 124 mRNAs coding for ribosomal protein subunits. This is done to promote synthesis of large amounts of protein chaperones that remove denatured proteins. Importantly, this transcriptional shut-down can be mimicked at 25°C using low amounts of thiolutin. Examination of deadenylation and decay rates showed that both processes are generally accelerated in response to stress. However, we could also determine that at any temperature decapping of most ribosomal protein-coding mRNAs, which constitute 40% of the transcriptome, is fully dependent on ongoing nuclear export. This sets this large group of mRNAs apart, and also shows that deadenylation is not the only key pre-requisite for decapping and decay.

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RNMT (RNA capping enzyme) complexes throughout T cell activation and differentiation

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Half the European population is currently expected to receive a cancer diagnosis within their lifetime. T cells (T lymphocytes) are a central part of the adaptive immune system, coordinating immune responses and mediating tumour cell killing. Modulation of RNA processing in T cells provides a novel opportunity to improve T cell responses to cancer. RNA pol II transcribed RNAs undergo co-transcriptional addition of an inverted guanosine to the 5' end to form an RNA cap structure. Methylation of this 5' RNA cap is a selective coordinator of gene expression. Previous work has demonstrated RNA cap methylation facilitates the metabolic rewiring of naïve T cells to give increased proliferation and cytoplasmic volume upon activation, most notably promoting ribosome production and mitochondrial remodelling. RNA guanosine N7 cap methyltransferase (RNMT) catalyses the addition of a methyl group to the inverted guanosine of the RNA cap at the N7 position. Here, we aim to understand the regulatory functions of different RNMT complexes in naïve, activated/effector, and memory T cells. So far, we have shown RNMT complexes are larger in activated T cells, as assessed by gel filtration, indicating increased protein binding, changes in protein structure, or RNA binding. We have also identified phosphorylation sites of RNMT in naïve and activated T cells by mass spectrometry. Future work will further characterize the different RNMT complexes found and elucidate how they facilitate changes in T cell metabolism and tumour cell killing, providing opportunity for therapeutic modulation.

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Developing a tool to uncover novel molecular targets in post-transcriptional latency of HIV – a genome-wide and targeted approach

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Antiretroviral therapy (ART) effectively suppresses the replication of Human Immunodeficiency Virus 1 (HIV-1). However, it is unable to completely eliminate the virus that remains dormant in long-lasting cellular reservoirs, which poses a major challenge to finding a cure. HIV-1 latency is mainly characterized at epigenetic and transcriptional levels, and little is known about post-transcriptional mechanisms and their contribution to reactivation. Recently, we have characterized a novel post-transcriptional block in latency linked to nuclear export of unspliced HIV-1 RNAs which is regulated by the viral protein Rev. Rev is a critical viral factor for productive viral infection and, thus, a critical component of replication-competent proviruses that are responsible for viral rebound. To this end, we have developed and characterized a dual-fluorescent HIV-1-based reporter system that tackles the

Rev-dependent export pathway. Our system distinguishes between Rev-independent (spliced, ECFP+) and Rev-dependent (unspliced, mKO2+) pathways, allowing for quantification of

Rev-dependent export efficiency by flow cytometry. Using this tool, we confirmed the effects of the known Rev cofactor MATR3 and the repressor CRNKL1 on Rev-dependent RNA export efficiency. We are now moving forward into a genome-wide CRISPR-based and

siRNA-mediated screening approaches to uncover novel cellular regulators of the

Rev-dependent pathway. Additionally, we are exploring the role of epitranscriptomic RNA modifications, including m6A, m5C, m1A, ac4C, and ψ in modulating HIV-1 RNA processing.

Identification and characterization of novel regulators of HIV-1 RNA nuclear export will expand our understanding about post-transcriptional mechanisms regulating viral gene expression and latency. Furthermore, it will provide new molecular targets for future therapeutic strategies aimed at eradicating HIV-1 reservoirs.

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The Role of RH11, RH37, and RH52 DEAD-box helicases in the regulation of microRNA biogenesis in plants

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In recent decades, the role of microRNAs (miRNAs) in plant development and physiology, as well as the mechanisms of their biogenesis, has garnered increasing scientific attention. MiRNAs are small non-coding RNAs that act as critical regulators of gene expression in eukaryotes. In plants, proper miRNA biogenesis is essential, and its complete disruption results in lethality. Primary miRNA transcripts (pri-miRNAs) contain stem-loop structures in which the mature miRNA sequence is embedded. Biogenesis occurs in two nuclear steps: first, the stem-loop is excised from the pri-miRNA to generate a precursor miRNA (pre-miRNA); second, a short miRNA/miRNA* duplex is produced from the pre-miRNA. Both steps are catalyzed by the RNase III enzyme DICER-LIKE1 (DCL1), with the assistance of two cofactors: the double-stranded RNA-binding protein HYPONASTIC LEAVES1 (HYL1) and the zinc finger protein SERRATE (SE).

In this study, we investigate the role of three DEAD-box RNA helicases—RH11, RH37, and RH52—in the regulation of miRNA biogenesis. Preliminary results from *Arabidopsis thaliana* mutants lacking these helicases revealed phenotypes resembling those of plants with elevated levels of mature miRNAs. Notably, RH11-FLAG overexpression lines exhibit a phenotype similar to the *hyl1-2* mutant, characterized by hyponastic leaves. Proteomic analysis demonstrated that several key proteins involved in miRNA processing, including SE, co-purify with the RH11-FLAG fusion protein. Furthermore, using confocal microscopy techniques, we confirmed nuclear co-localization of RH11 and SE.

Additionally, developmental phenotypes such as delayed flowering were observed in *rh11 x rh37* and *rh37 x rh52* double mutants, underscoring the biological importance of these helicases. Based on our findings, we propose a model in which RH11, RH37, and RH52 act as negative regulators of miRNA biogenesis. We hypothesize that these helicases influence the secondary structure of pri-miRNAs, thereby preventing efficient binding of the DCL1/HYL1 complex and subsequent processing into mature miRNAs.

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Functional and interactome analysis of N6-adenosine methyltransferase (MpMTA) in *Marchantia polymorpha*

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N6-methyladenosine (m^6A) is the most common post-transcriptional RNA modification, found in mRNAs, tRNAs, rRNAs, and various non-coding RNAs. This modification influences key aspects of RNA metabolism, including transcript stability, translation efficiency, nuclear export, 3'-end processing, and alternative splicing. In addition, m^6A has been shown to stabilize pri-miRNAs, thereby promoting their proper processing into mature miRNAs. The m^6A mark is catalyzed by a multicomponent methyltransferase complex, in which the MTA (N6-adenosine methyltransferase) serves as the core catalytic subunit responsible for the transfer of methyl groups onto RNA molecules. While the significance of m^6A is well documented in angiosperms such as *Arabidopsis thaliana*, its role in early-diverging land plants, including the liverwort *Marchantia polymorpha*, remains poorly understood.

In this study, we investigated the effects of knockout of Mp*MTA* (Mp*Δmta*) and its overexpression (MpMTA^{OE}) on pri-miRNA expression and mature miRNA accumulation, focusing on both conserved (shared across land plants) and *Marchantia*-specific miRNAs. Our results show that MpMTA does not significantly affect selected pri-miRNA levels or miRNA accumulation in *M. polymorpha*.

To explore the protein interactome of MpMTA, we performed co-immunoprecipitation followed by mass spectrometry (MS) in Mp$MTA\text{-}3xCit^{OE}$ transgenic plants. These analyses revealed that MpMTA interacts with a broad range of proteins, including RNA and DNA methyltransferases, splicing factors, helicases, transcriptional and translational regulators, and other RNA-processing components.

Additionally, we characterized the phenotypes of wild-type, Mp$MTA\text{-}3xCit^{OE}$, and Mp*Δmta* plants, with particular emphasis on thallus development and the structure of antheridiophores—male reproductive organs that exhibit high *MTA* expression. Mp*Δmta* mutants displayed delayed development of gemma cups and morphological alterations in antheridiophores, including modified architecture of the stalk's upper region. In contrast, Mp$MTA\text{-}3xCit^{OE}$ plants exhibited no major differences in thallus morphology, but their antheridiophores showed protruding, solitary antheridia.

P145

BRM, a SWI/SNF ATPase controls *Arabidopsis thaliana* secondary seed dormancy through a long non-coding antisense transcript of *DOG1*

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The *DOG1 (Delay of Germination 1)* gene is a main regulator of seed dormancy and germination in plants. Seed dormancy is generally categorized into primary dormancy, established during seed maturation, and secondary dormancy, which is induced when seeds encounter unfavorable environmental conditions during imbibition. Our recent studies have highlighted the role of the SWI/SNF chromatin remodeling complex, particularly the BRM ATPase subunit, in modulating *DOG1* expression during secondary dormancy induction. Using darkness and high-temperature treatments to induce secondary dormancy, we have shown that mutations in the BRM ATPase lead to increased dormancy phenotypes. Molecular analyses, including ChIP and RT-qPCR, revealed that BRM regulates *DOG1* gene expression through antisense transcription mechanisms. Our data show that the antisense transcript, *asDOG1*, functions as a negative regulator of dormancy, and its expression is decreased in *brm* mutant seeds during secondary dormancy induction.

Our model suggested that BRM-containing the SWI/SNF complex binds to the 3' end region of *DOG1* and, under secondary dormancy-inducing conditions remodels nucleosomes to activate *asDOG1* antisense promoter. This activation results in the production of *asDOG1* transcripts, which suppress *DOG1* activation and ultimately promote the establishment of strong dormancy.

I will discuss our new results showing that a nuclear RNA 5'–3' exonuclease XRN3 involved in transcription termination is also involved in secondary dormancy regulation. We hypothesise that *asDOG1* transcription termination regulation is another layer of *DOG1* regulation during secondary seed dormancy establishment.

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Chromatin-Mediated Regulation of *DOG1* Antisense Transcription in Arabidopsis Seeds

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Seed dormancy in *Arabidopsis thaliana* is critically regulated by the DELAY OF GERMINATION1 (DOG1) gene. Our studies have identified an antisense transcript, asDOG1, originating from the 3' end of DOG1, which negatively regulates DOG1 expression[1]. The chromatin remodeler BRAHMA (BRM) and the DNA repair protein BRCA2A have been implicated in modulating antisense transcription at the *DOG1* locus, suggesting a complex interplay between chromatin dynamics, DNA damage and antisense regulation[2].

To dissect the regulatory elements and factors controlling *asDOG1* expression, we employed a multifaceted methodological approach. Initially, we performed transient FAST expression assays in Arabidopsis seedlings using GFP reporter constructs containing various fragments of the *DOG1* 3' region. Subsequently, we utilized Self-Transcribing Active Regulatory Region sequencing (STARR-seq) to map enhancer elements within the *DOG1* locus, revealing regions with high enhancer activity correlating with *asDOG1* expression. These assays identified minimal promoter regions capable of driving *DOG1* antisense transcription.

To capture the dynamics of transcription at the *DOG1* locus, we conducted Native Elongating Transcript sequencing (NET-seq), which provided high-resolution profiles of nascent transcription[3]. NET-seq data indicated that BRM influence the transcriptional landscape of the *DOG1* locus, affecting sense transcription through its antisense and splicing regulation[2]. To validate the functional relevance of identified regulatory elements, we generated CRISPR/Cas9-mediated deletions targeting putative antisense promoter regions. Analysis of these mutants demonstrated altered *DOG1/asDOG1* expression levels and corresponding changes in seed dormancy phenotypes.

Our integrative approach combining transient expression assays, STARR-seq, NET-seq, and CRISPR-mediated genome editing elucidates the complex regulatory network governing *DOG1* antisense transcription. These findings highlight the pivotal roles of BRM and BRCA2A in modulating chromatin structure and transcriptional regulation at the *DOG1* locus, providing insights into the epigenetic control of seed dormancy.

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P147

Spatial expression patterns of miRNAs across different anatomical regions and cell types of the pituitary gland

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A few individual miRNAs have been shown to play an essential role in the development and function of endocrine tissues, and these have been studied mainly for the pancreas in the context of insulin secretion and the pituitary gland (PG). PG consists of 6 main types of endocrine cell types, each devoted to producing and secreting different peptide hormones, and a few non-endocrine cell types, including pituicytes and stem cells. It remains unsolved whether miRNA regulation and miRNA themselves are expressed in a cell-type-specific manner in these different endocrine cell populations. Here, we aimed to find out if there is any specificity in miRNA expression patterns across discrete pituitary anatomical regions and their distinct cell type populations. We used RNAscope assay and Qupath software to evaluate and quantify miRNA signal across anterior (AL), posterior (PL), and intermediate (IL) lobes, as well as the marginal zone that is known to be populated by pituitary stem cells. By incorporating mRNA markers, we were able to analyze miRNA signal across individual cell types: somatotropes, gonadotropes, corticotropes, melanotropes and stem cells. We analyzed the top two highest expressed miRNAs in PG (miR-7 and miR-375) and identified both of them to be broadly and highly expressed within the endocrine part of the PG. Three other high-to-medium expressed: miR-30c, miR-129-2-3p and miR-183, had distinct expression patterns. miR-129-2-3p has been found predominantly in the IL, and its melanotrope cells. In turn, neither miR-30c nor miR-183 has been region-specific. miR-30c showed enrichment in corticotropes, while miR-183 exhibits a bimodal expression pattern (ON/OFF) in all studied cell types. We also found that miRNA quantities vary across Sox2-positive cells depending on their anatomical location. Jointly, our results show that miRNAs which we have been studying are rather enriched than cell-type-specific in different regions or cell types in PG.

P148

Ribosomal protein Rps29/uS14 contributes to 18S rRNA maturation and its abundance regulates osmotic stress response in S. cerevisiae

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The ribosome is the universal machine for protein synthesis across all life. All ribosomes consist of a conserved core of ribosomal proteins and RNAs (rRNAs) that mediate accurate decoding of mRNAs to synthesize functional proteins. This macromolecular complex is composed of two subunits: 60S—a large subunit (LSU), and 40S—a small subunit (SSU). Assembly of these ribosomal subunits is highly regulated, with several well-characterized quality control checkpoints before they enter into the translating pool of ribosomes. The 40S as part of this complex plays essential roles in assembling and stabilizing ribosomes, which contain one rRNA (18S) and 33 ribosomal proteins (RPs). Therefore, the accurate assembly and maturation of the 40S subunit, is one of the most energy-intensive cellular processes and must be coordinated precisely.

One of the ribosomal proteins of interest is RPS29, which has been implicated in Diamond-Blackfan Anemia (DBA), a rare genetic disorder characterized by impaired production of red blood cells. However, the specific contributions of RPS29 to ribosome heterogeneity and cellular function remain poorly understood.

In this study, we investigate the function of Rps29 (uS14). In Saccharomyces cerevisiae, two highly similar paralogous genes, RPS29A and RPS29B, which arose from gene duplication and share 91% sequence identity, encode Rps29 protein. By individually deleting each paralog, we systematically assessed their roles in ribosome assembly, rRNA processing, and translation efficiency. Our findings reveal that Rps29 is crucial for pre-rRNA maturation and the integrity of the 40S subunit. Furthermore, our data have shown that the deletion of either paralog altered cellular responses to hyperosmotic stress and improved growth, suggesting decreased levels of Rps29 might be an adaptive advantage to sustain yeast under such conditions. Notably, we observed a significant increase in the level of activation of Hog1 MAPK upon hyperosmotic stress, reflected in the ribosome biogenesis and translational patterns. These results provide new insights into ribosome specialization and highlight how ribosomal protein composition can quickly change the landscape of cells' responses to environmental stress.

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P149

Impact of N6-methyladenosine mark on molecular response to heat stress in *Arabidopsis thaliana*

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High temperatures are among the most common abiotic factors that negatively impact plant growth and development. A key player in the regulation of the plant stress responses is N6-methyladenosine (m6A), which is the most prevalent internal epitranscriptomic modification in eukaryotic mRNAs. In plant cells it has been observed that changes in the m6A methylation level significantly affect stress response mechanisms. Previous studies suggest that the presence of the m6A mark in transcripts influences the early plant response to low temperature stress (4°C), causing reprogramming of the expression of many genes and altering the kinetics of the RNA Polymerase II (RNAPII) complex within methylated bases. It has been suggested that m6A may have a similar effect on the plant response to high temperature stress, but it probably affects the expression of other sets of genes.

The aim of presented work is to comprehensively analyse the initial transcriptomic response of *Arabidopsis thaliana* to environmental high temperature stress and to investigate its effects on plant development. The approach presented in this project involves a combination of plaNET-seq, RNA-seq and PAT-seq techniques to investigate dynamic transcriptomic changes during heat stress exposure. The RNA-seq technique provides a comprehensive understanding of gene expression in heat-stressed and nonstressed plants under steady-state conditions but does not allow all parameters to be distinguished. The plaNET-seq enables the isolation of transcripts actively transcribed by RNAPII by immunoprecipitation of RNAPII complexes and subsequent isolation from RNA from these complexes. On the other hand, the PAT-seq technique, allows sequencing of the 3' ends of the mRNA.

The following project focuses on how the early transcriptomic response of plants changes under high temperature stress and how the lack of the m6A mark affects this response. The multilateral analysis of the transcriptome performed in this project will provide a comprehensive picture of mRNA kinetics under high temperature and will enable a model to be proposed to illustrate the effects of short-term high-temperature stress on RNA metabolism, with particular emphasis on the relationship with m6A marks and how they affect mRNA stability. Preliminary studies on the role of N6-methyladenosine mark will be presented.

P150

Formation and Dynamics of Nuclear Foci Containing Toxic RNA with Expanded CUG Repeats in Cells with Inducible Expression of Fluorescently Tagged MBNL Isoforms

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Myotonic Dystrophy type 1 (DM1) is a neuromuscular genetic disorder caused by CTG repeat expansion within the untranslated region of the *DMPK* gene. Mutant transcripts harbor expanded CUG repeats (CUGexp) which interact with and sequester MBNL (Muscleblind-like) protein family, leading to their accumulation within nuclear foci of skeletal and heart muscles as well as neural cells. MBNLs are regulators of cellular RNA metabolism including alternative splicing, therefore sequestration in foci affects their functional level. The question arises what are other factors influencing *CUGexp* foci formation. In this work, we present our approach wherein exogenous MBNL1 splicing variants will be expressed upon doxycycline treatment with cumate induced expression of mutant *DMPK* minigene, in double knock-out cells (MBNL1 and MBNL2 KO). The transgenic MBNL proteins will be tagged with fluorescence proteins, while *DMPK* minigene containing 960 CUG repeats will be aptamer tagged, which binds fluorophore. This will enable to observe the properties of foci formation, including their number, size, morphology, and dynamics, under various conditions such as the presence or absence of different MBNL isoforms.

P151

Downregulation of CWC22 splicing factor leads to global changes in gene expression and splicing stimulating renal cell carcinoma cell migration

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Dysregulation of alternative splicing (AS), observed in cancer, disrupts key genes involved in tumorigenesis, often due to aberrant splicing factor expression. Renal cell carcinoma (RCC), the most prevalent kidney malignancy, exhibits significant AS alterations, with underlying causes remaining largely unknown.

Our analysis revealed that the expression of the splicing factor CWC22 is significantly reduced in advanced stages of renal cell carcinoma (RCC), and its diminished expression is associated with unfavorable patient survival outcomes CWC22 plays a critical role in stabilizing 5' exons prior to the first splicing step and positioning the exon junction complex (EJC) on mRNA, thereby influencing splicing, mRNA export, nonsense-mediated decay and translation.

To investigate the functional significance of CWC22 in RCC, we silenced its expression using siRNA in three cell lines: 786-O and A498 (derived from primary tumors), and Caki-1 (derived from a metastatic lesion), which together represent the heterogeneity of clear cell RCC (ccRCC). Microarray analysis showed that CWC22 silencing led to substantial transcriptomic changes, with differentially expressed genes (DEGs) numbering 3321, 2715, and 2274 in 786-O, A498, and Caki-1 cells, respectively. Among 429 common DEGs, we identified oncogenic drivers such as HIF1A and genes involved in mRNA processing (QKI, SF3B3, SKP2, and MERCKS). Ingenuity Pathway Analysis (IPA) of DEGs revealed enhanced tumor cell migration and motility in all cases, while proliferation was mildly or not activated in 786-O and Caki-1, and inhibited in A498.

Even more profound alternations were observed in splicing, over 5000 transcripts underwent AS across all models following CWC22 depletion, with pathway enrichment analysis implicating Rho GTPase signaling and cell cycle regulation. Transcriptomic shifts and AS events in 786-O cells were further characterized via direct RNA sequencing using Nanopore technology. Cellular functional assays demonstrated that CWC22 downregulation significantly increased the motility of 786-O cancer cells, while moderately reducing proliferation and viability. This findings are consistent with the results of the transcriptomic analysis. Our findings identify CWC22 as a crucial regulator of both transcription and AS in RCC cells. IPA and enrichment analyses revealed alterations in critical cellular processes, linking the loss of CWC22 to tumor progression through enhanced cell migration and its potential role in metastasis.

P152

miR-28-5p as a regulator of rhabdomyosarcoma development

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MicroRNAs (miRNAs) are a class of small non-coding RNA molecules that play a crucial role in the post-transcriptional regulation of gene expression. MiRNAs play important roles in development of different tumor types including rhabdomyosarcoma (RMS). RMS is one of the most prevalent soft tissue sarcoma in children and adolescents with a remarkably low survival rate depending on the subtype of RMS. The pathogenesis of this malignant tumor is associated with an impaired differentiation of stem cells or myogenic progenitor cells. The SNAIL transcription factor is a critical regulator of RMS development, and it affects levels of miRNAs, including miR-28-5p.

The aim of the study was to investigate the role of miR-28-5p in different processes regulating RMS development: proliferation, migration and myogenic differentiation.

In this study, alveolar (RH30, RH41) and embryonal (RD) subtypes of RMS were transfected with miR-28-5p mimics. The effect of miR-28-5p transfection was evaluated by molecular biology techniques and assays. The results showed that miR-28-5p increased levels of myogenic regulatory factors (MRF) such as MYOD, MYOG and MSTN. Furthermore, it decreased proliferation of RMS cells and regulated cell cycle and BrdU incorporation. MiR-28-5p also reduced migration of RMS cells in scratch assay.

To summarize, miR-28-5p plays a pivotal role as a regulatory factor of proliferation, migration and myogenic differentiation of RMS cells *in vitro*.

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P153

Characterization of the IFIT proteins role in innate immunity

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IFITs (interferon-induced proteins with tetratricopeptide repeats) are a family of RNA-binding proteins, known for their role in the innate immune responses in vertebrates. Their expression is induced in response to interferon, viral infection, or recognition of PAMP (pathogen-associated molecular pattern). In humans, the IFIT family consists of five paralogues - IFIT1, IFIT1B, IFIT2, IFIT3 and IFIT5. Among IFIT proteins, only IFIT5 occurs as a monomer, while IFIT1, IFIT2 and IFIT3 form homo- or hetero-complexes with themselves. Antiviral properties of IFITs originate from their ability to discriminate foreign RNA, based on its molecular patterns. The molecular patterns recognized by IFITs include cap0 (IFIT1) or AU-rich sequences (IFIT2), that may resemble AU-Rich Elements (AREs).

In our research, we investigate the antiviral and RNA regulatory functions of IFIT proteins. To identify RNA molecular partners of IFIT2, we co-expressed and purified a recombinant IFIT2/3 heterodimer, which we employed as bait in RNA pull-down assays. Interestingly, this approach revealed selective enrichment of IFIT mRNAs, suggesting a potential autoregulatory mechanism whereby IFIT proteins may bind and modulate the expression of their own transcripts. To explore the role of IFIT proteins in the regulation of host mRNAs, we optimized the iCLIP2 protocol for 3xFLAG-tagged IFIT1 in A549 cells. This method enables high-resolution mapping of RNA–protein interactions and lays the foundation for transcriptome-wide identification of IFIT1-bound RNAs under physiological or stimulated conditions. In parallel, we investigated the role of IFIT proteins in the context of viral infection. Using an A549 cell line with a knockout of the IFIT3 gene, we observed a significant increase in Sindbis virus (SINV) replication compared to wild-type cells. These findings uncover a previously unrecognized antiviral function of IFIT3.

P154

Small RNA MTS1338-mediated virulence of mycobacterium tuberculosis

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MTS1338, a distinctive small non-coding RNA (sRNA) in pathogenic mycobacteria, plays a crucial role in host-pathogen interactions during infection. Mycobacterial cells encounter heterogeneous stresses in macrophages upon infection. MTS1338 has been implicated to be highly abundant in response to a series of stress conditions such as low pH, H_2O_2, hypoxia, and NO. The dormancy regulatory factor DosR is a two-component system regulating ~50 genes in the Mycobacteria, only recently delineated to be involved in MTS1338 abundance. Little is known about sRNA-mediated regulation in M. tuberculosis. We have taken a biochemical approach and identified that DosR can strongly bind to the two regions upstream of the MTS1338 gene (DrrS). The proximal region, though individually, possesses a 3-fold higher affinity than the distal site; however, the presence of both regions increased the affinity of the MTS1338 gene for DosR by >10-fold due to cooperative binding. Although PhoP did not directly bind to the MTS1338 gene, it binds to the DosR-bound MTS1338 gene, which is evocative of a concerted regulation of MTS1338 expression under stresses. PhoP regulates >100 genes as a transcription factor and controls whiB6 (Rv3862c) and other pH-responsive genes in Mycobacterium tuberculosis. Understanding the gene regulatory networks in Mtb is an important and daunting task since WhiB6 regulates the DosR regulon and ESX-1 differently, affecting virulence factor (EsxA and EsxB heterodimer) secretion and pathogenicity (ESAT-6). MTS1338 sRNA directly binds with the whiB6 mRNA transcript at its 3' end (19 bp), likely safeguarding it against 3' ribonucleases. This interaction may enhance the half-life (5-6 h) of MTS1338, i.e., Mycobacterium tuberculosis small RNA. The whiB6 expression upregulated 4-fold under acid (pH-4.5), 5-fold under tBHP oxidative stress, and ~10-fold under the MTS1338 overexpressing H37Ra compared to the wild-type. This research highlights the PhoP network that is a part of sRNA regulation at low pH and may significantly aid in developing novel pharmaceuticals targeting the small RNA MTS1338 and whiB6 transcript interactions. Overall, This study substantially adds to our knowledge of the virulence regulation of MTS1338 in Mycobacterium tuberculosis.

Keywords: Mycobacterial Small RNA; Dormancy regulatory factor; Cooperative binding; Virulence factor secretion; WhiB6 redox sensor.

P155

Activation of cell stress response pathways as a source of Ribosomal Inactivating Proteins cytotoxicity

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One of the most well-known toxic proteins are ribosome inactivating proteins (RIPs) produced mainly by plants and bacteria. These toxins are a threat to human life and pose a high social risk due to the fact that they could be used in chemical warfare. Due to their toxic potency, RIPs have also attracted attention in medicine, in particular for the development of targeted anti-viral and anti-cancer therapy. RIPs are categorized into three primary classes, Type I, Type II, and Type III, based on their architecture and mode of action. Type I RIPs, such as trichosanthin (TCS), are single-chain proteins. In contrast, Type II RIPs, including ricin and Shiga toxin, are composed of two distinct components: an enzymatically active A subunit and a B subunit with lectin-like properties that mediates cellular uptake, linked by a disulfide bridge. Type III RIPs are synthesized as inactive precursors that must undergo proteolytic processing to become active. Despite extensive research, the detailed molecular pathways underlying RIP-induced toxicity remain incompletely defined. The most widely accepted hypothesis posits that RIPs function as RNA N-glycosidases, specifically targeting and depurinating the sarcin-ricin loop (SRL) of ribosomal RNA. The SRL, located in the GTPase-associated center (GAC) of the ribosome, is essential for facilitating GTP hydrolysis by translation-associated GTPases. Damage to this critical region impairs ribosomal function, inhibits protein synthesis, and is consudered to trigger apoptotic cell death. Here, we employed a range of biochemical and cellular assays to analyze representatives from each RIPs category. Our results reveal, that SRL depurination by RIPs initiates stress response pathways in mammalian cells. We can propose that rather than protein synthesis inhibition being the direct cause of cell death, our data support a model in which RIP-induced ribosomal damage activates the pervasive ribotoxic stress response (RSR). This, in turn, engages downstream signaling pathways that culminate results in apoptosis.

P156

FUS/TLS as a potential regulator of PABPN1 in skeletal muscle?

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Poly(A) binding protein nuclear 1 (PABPN1) is an ubiquitous polyadenylation factor that activates the poly(A) polymerase (PAP) and controls poly(A) tail length of mRNA.

A short expansion in N-terminal of the protein leads to oculopharyngeal muscular dystrophy (OPMD), a rare genetic muscle disease characterized by ptosis and dysphagia. In OPMD muscles, expanded PABPN1 aggregates lead to a loss of functional PABPN1 (Roth et al Acta Neuropath 2022). Compared to any other tissue, PABPN1 protein level is very low in skeletal muscle, and even lower in pharyngeal muscles. Depletion of PABPN1 in vivo in mature skeletal muscle leads to muscle degeneration, atrophy and altered RNA metabolism. PABPN1 level is also down-regulated during muscle aging.

Despite its critical role in mRNA processing, the mechanisms regulating PABPN1 expression in human skeletal muscle remain poorly understood, particularly in the context of OPMD.

In this context, using a comprehensive database that analyzes high-throughput sequencing data from cross-linking and immunoprecipitation (CLIP-seq) available datasets, we selected the RNA binding proteins (RBP) FUS/TLS as a potential regulator of PABPN1 expression.

Using siRNA and/or overexpression systems, we tested FUS/TLS as a regulator of PABPN1, both in vitro in human muscle cell lines and in vivo by AAV intramuscular injection in mice.

We showed that FUS/TLS depletion leads to the downregulation of PABPN1 expression at the RNA and protein level both in vitro and in vivo.

We confirmed FUS/TLS protein binding to PABPN1 mRNA by RNA immunoprecipitation and

mechanistic analyses are on-going to understand how FUS/TLS regulates PABPN1 mRNA.

Altogether, our data identify FUS/TLS as a novel post-transcriptional regulator of PABPN1 and suggest it as a molecular target to modulate disease progression in OPMD and improve muscle ageing.

P157

m6A Dynamics in Head and Neck Cancer: HPV-Driven Epigenetic Divergence Revealed by Patient Data and In Vitro Experiments

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Background: N6-methyladenosine (m6A), as the most prevalent and dynamic mRNA epigenetic modification, plays a crucial role in various biological processes and together with HPV, has a significant impact on HNSCC development. HPV-negative HNSCC arises from prolonged exposure to carcinogens, leading to genomic instability and somatic mutations. In contrast, HPV-positive HNSCC patients tend to have better overall survival and higher response to radio-/chemotherapy, likely due to preserved DNA repair pathways and fewer TP53 mutations.

Aim of the study: This study aims to elucidate the role of RNA methylation in HNSCC HPV-driven tumorigenesis and the dynamic nature of m6A modification.

Material&Methods: We used RT-qPCR and Western-Blot to perform gene expression analysis of METTL3, VIRMA, FTO, ALKBH5, YTHDF2, YTHDF3, YTHDC1, YTHDC2 on 87 HNSCC patients and 5 HPV-negative (FaDu, H103, Detroit-562, SCC-9, SCC-25) and 2 HPV-positive (SCC-152, SCC-154) cell lines. Global m^6A RNA methylation abundance in HPV-negative and HPV-positive in vitro models was quantified by colorimetric assay. We performed RNA-seq of 2 HPV- positive (SSC-152, SSC-154) and 2 HPV-negative (SSC-9, SSC-25) cell lines to obtain complex transcriptomic profiles of HNSCC subtypes.

Results: HNSCC cancer tissue exhibits lower mRNA expression of m6A Writer METTL3, m6A Eraser FTO, and m6A Readers YTHDC2, YTHDF2 genes compared to normal tissue. Time-dependent dynamic changes in m6A RNA methylation machinery were revealed in Fadu, H103, and Detroit-562 cell lines. Global m6A abundance as well as m6A Writer VIRMA and m6A Reader YTHDC2 mRNA expression were higher in HPV-positive cell lines. The RNA-seq analysis highlights differential correlation patterns of m6A regulators depending on HPV status.

Conclusion: Our study based on HNSCC patients' material and in vitro models indicates the high involvement of m6A RNA methylation in both HPV-negative and HPV-driven tumorigenesis. We have also underlined the time-dependent nature of these processes and their high clinical potential. Our data suggests gene-specific involvement of m6A machinery in repressing expression of selected targets, with notable differences between HPV-positive and HPV-negative tumors.

P158

Elucidating the interplay between m6A and lncRNAs in breast cancer

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N6-methyladenosine (m6A) is one of the most prevalent modifications in eukaryotic mRNAs and long non-coding RNAs (lncRNAs), playing a crucial role in post-transcriptional gene regulation. Emerging evidence suggests that m6A modifications influence RNA stability, cellular localization, splicing, and translation, ultimately affecting various biological processes and disease states. m6A is regulated by "writers" (methyltransferases, e.g., METTL3/14), "erasers" (demethylases, e.g., FTO, ALKBH5), and "readers" (e.g., YTHDF proteins) that determine its fate and functions. In cancer, m6A-modified lncRNAs act as oncogenes or tumor suppressors by regulating signaling pathways such as PI3K-AKT and Wnt/β-catenin. Advances in m6A detection technologies, such as MeRIP-seq, have deepened our understanding of m6A's role; however, all antibody-dependent techniques carry some limitations, e.g., low reproducibility and resolution. To overcome these challenges, we developed a novel antibody-independent assay for mapping m6A modifications in lncRNAs. By applying this approach in breast cancer cells, we were able to achieve high-precision detection of m6A-modified sites without the drawbacks associated with traditional immunoprecipitation-based techniques. Following the identification of m6A-modified lncRNAs, we performed functional characterization studies to elucidate their roles in breast cancer development and progression. Our findings provide new insights into the regulatory landscape of m6A in cancer and highlight potential therapeutic targets.

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Identification of m6A modification on selected lncRNAs in breast cancer.

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N6-methyladenosine (m6A) is one of the most prevalent RNA modifications and plays a key role in regulating gene expression. In addition to messenger RNA (mRNA), it has been shown that other types of RNA, including long non-coding RNAs (lncRNAs), are m6A-modified. LncRNAs are transcripts longer than 200 nucleotides that do not encode proteins. The goal of our study is to identify the precise locations and potential functions of specific m6A sites on cancer-related lncRNAs. In the first part of the study, we used quantitative PCR (qPCR) to confirm the expression levels of m6A writers, including METTL3, in selected breast cell lines. To further explore the role of m6A, we established a stable MCF-7 cell line with reduced METTL3 expression using short hairpin RNA (shRNA)-mediated knockdown. This cell line will enable us to further investigate the presence and role of m6A modification in the lncRNAs of interest. Furthermore, we plan to identify the precise locations of m6A marks on specific lncRNAs using a novel technique involving 4SedTTP reverse transcription.

P160

The influence of HIV sense- and antisense transcripts on stochastic HIV gene expression and reactivation

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Human immunodeficiency virus (HIV) has two promoters, 5' and 3' long terminal repeats (LTRs), that govern sense- and antisense transcription, respectively. Accumulating evidence supports that HIV antisense transcripts (ASTs) can suppress sense RNA transcription *in vitro* and *in vivo*; however, whether HIV ASTs contribute to stochastic fluctuations of HIV gene expression, and if they do, the mechanistic competition between sense- and antisense transcripts is not fully understood. In this study, we established dozens of single provirus-infected cellular clones offering various transcriptional phenotypes of HIV. We proposed that stochastic fluctuations of HIV gene expression can appear at least at two levels: the level of (1) the chromosomal landscape and (2) the site of integrated HIV. In the former case, proviruses integrating in different genomic locations demonstrated a variety of transcriptional bursting that negatively correlated with provirus transcription. Proviruses possessing varying levels of stochastic fluctuations in transcription can be classified in noise space constructed by parameters associated with coefficient of variation or by using a mathematical model fitting a curve of exponential decay proposed in this work. In the latter case, stochastic HIV gene expression tended to be a pure epigenetic phenomenon: the turnover of the state of infection showed an elevated frequency from the identical provirus. We identified the integration site of such a provirus, which is present in the vicinity of the genomic repetitive regions (SINEs and Alu elements) associated with intense signals of H3K27ac and H3K4me3. In both scenarios, we observed a similar rhythm of consecutive expression patterns and a positive correlation between the abundance of provirus sense- and antisense RNA transcripts in time series, suggesting that the independent yields of both transcripts determine the stochastic phenotype of provirus transcription in our cellular models instead of their ratios. Notably, although the abundance of antisense transcripts was less than that of sense RNAs overall, different affinities between two HIV LTRs were observed across different cellular models in the presence of different drugs. Overall, our data suggested that HIV ASTs could be involved in the stochastic nature of its transcription.

P161

Exploring the Hepatoprotective Mechanisms of Curcuma longa: Evidence from Porcine and Human Systems

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Curcuma longa, commonly known as turmeric, is a renowned medicinal herb recognized for its hepatoprotective and gastrointestinal benefits, particularly in counteracting the deleterious effects of poor dietary habits. The principal bioactive compound, curcumin, exhibits significant regulatory potential over gene expression involved in critical biological and metabolic pathways related to detoxification, oxidative stress mitigation, and inflammatory control. Given its multifaceted pharmacological properties, curcumin is increasingly being studied for its molecular mechanisms in the prevention and management of hepatic dysfunction. Transcriptomic analyses are essential to comprehensively understand the hepatoprotective actions of Curcuma longa at the molecular level. Young pigs, due to their highly responsive immune systems and physiological similarities to humans, serve as an ideal model for investigating these effects. In a forthcoming study, immediately following slaughter, a wide array of vital organs and tissue segments were collected from all experimental swine (n = 154), including the liver, kidneys, heart, pancreas, spleen, pituitary gland, stomach (ventriculus), and contents of the gastrointestinal tract (duodenum, jejunum, ileum, cecum, colon), along with blood serum obtained using both heparin and EDTA. Comprehensive haematological and biochemical analyses were performed on blood samples drawn via venipuncture from the external jugular vein (vena jugularis externa) before slaughter and repeated post-mortem. These evaluations, conducted at the Municipal Hospital Laboratory in Olsztyn, encompassed a broad range of

parameters, including WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, NEUT, LYM, MONO, EOS, BASO, as well as immunoglobulin classes (IgA, IgE, IgM, IgG), and standard biochemical markers such as TC, HDL, LDL, TG, GLU, ALT, AST, ALP, BIL, and GGTP. Our preliminary findings demonstrated a significant protective effect of CL against AFB1-induced alterations in pigs, specifically reflected in the blood parameters lymphocyte and neutrophil counts.

Our preliminary results concludes that the genetic and physiological congruence between pigs and humans further underscores the utility of the porcine model in exploring gene expression changes relevant to human health. This enables the identification of therapeutic targets and supports the development of evidence-based interventions for liver diseases. Integrating these findings into modern clinical practice holds promise not only for advancing our understanding of curcumin's protective functions but also for improving outcomes in individuals suffering from chronic hepatic conditions and malignancies that continue to pose significant global health and economic burdens.

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SplicEx – a User-Friendly Framework for Simplifying the Functional Interpretation of Alternative Splicing Events

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Alternative splicing of precursor mRNA is a critical regulatory mechanism that contributes to transcriptomic and proteomic diversity, with its dysregulation implicated in a broad spectrum of human diseases. Despite the availability of numerous computational tools, the comprehensive characterization of splicing events and their molecular consequences remains a significant and labour-intensive challenge, largely due to the complexity of integrating multi-omic data and diverse layers of functional annotation.

Here, we introduce SplicEx, a computational pipeline designed to systematically identify and functionally characterize alternative splicing events. SplicEx builds on the outputs of established RNA-seq splicing detection tools such as rMATS and SplAdder, applying statistical filters to prioritize high-confidence events. These events are then annotated in terms of their transcript-specific effects, including reading frame disruptions, structural changes in protein-coding sequences or untranslated regions (UTRs), and alterations in domain architecture. A distinctive feature of SplicEx is the integration of protein sequence data with transcript exon structure, enabling precise mapping of splicing events relative to the resulting protein and facilitating a systematic classification of their structural and functional impacts.

The pipeline further incorporates Gene Ontology enrichment analysis to identify affected biological pathways, and produces intuitive visualizations of splicing events, transcript isoforms, and altered protein domains. All components of the pipeline are interconnected, allowing comprehensive exploration of the functional implications of alternative splicing events. Additionally, a beta version of a web-based SplicEx interface enables users to perform analyses without the need for manual tool configuration.

To demonstrate SplicEx's utility, we applied it to RNA-seq data from a study of RNA polymerase III-related hypomyelinating leukodystrophy (HLD). Our analysis revealed disease-associated splicing defects that emerge as strong candidates for contributing to the HLD phenotype.

In summary, SplicEx provides a user-friendly and integrated framework that bridges splicing detection with functional interpretation, facilitating the discovery of splicing-driven mechanisms in disease.

Keywords: alternative splicing, transcriptomics, spliceosome, leukodystrophy, RNAPIII, RNA-seq, bioinformatics